XX.—*On the Sources of the Nitrogen of Vegetation; with special reference to the question whether Plants Assimilate Free or Uncombined Nitrogen.*

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WITH our present knowledge of the general composition of plants, and of the sources of their constituents, it is easy to see how essential was a proper understanding of the chemistry of the air, and of water, to any true conception of the material changes involved in the vegetative process. It was in fact between 1770 and 1800, that Black, Scheele, Priestly, Lavoisier, Cavendish, and Watt, were engaged in establishing that common air consists chiefly of nitrogen and oxygen, with a little carbonic acid,—that carbonic acid is composed of carbon and oxygen,—and water of hydrogen and oxygen;—and it was within the same period, that Priestly and Ingenhousz, Sennebier and Woodhouse, laboured to show the mutual relations of these bodies and vegetable growth. They seem, however, to have had more prominently in view the question of the influence of plants upon the media with which they were surrounded, than that of the influence of those media in contributing to the increased substance of plants themselves.

Following closely on the footsteps of the above observers, both

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Supplied with no other combined Nitrogen than that contained in the seed sown.

Wheat 1857
in prepared soil Barley 1857
in prepared soil Barley 1857
in prepared punnet Wheat 1858
in prepared soil Barley 1858
in prepared soil Oats 1858
in prepared soil

Fig 14

Fig 15

Fig 16

Fig 17

Supplied with known and limited quantities of combined Nitrogen beyond that contained in the seed sown.

Wheat 1857
in prepared soil Barley 1857
in prepared soil Barley 1857
in prepared punnet Wheat 1858
in prepared soil Barley 1858
in prepared soil prepared Oats 1858
in prepared soil Wheat Barley & Beans 1857
in garden soil

in point of time and general plan of research, came De Saussure, whose labours were conducted towards the end of the last, and in the beginning of the present century; and their results together with the arguments founded upon them, were published by him in 1804. To De Saussure we owe the experimental, and even quantitative, illustration of the fact, that plants, in sunlight, increase in their amounts of carbon, hydrogen, and oxygen, at the expense of carbonic acid and of water. Indeed, he concluded that air and water contributed a much larger proportion of the dry substance of plants, than did the soils in which they grew.

Besides carbon, hydrogen, and oxygen, plants had already been shown to contain *nitrogen*. Priestly and Ingenhousz thought they had observed that plants absorbed the free nitrogen of the confined atmospheres in which they were placed in their experiments. Sennebier and Woodhouse arrived at an opposite conclusion. De Saussure, again, thought that his experiments indicated rather an evolution of nitrogen at the expense of the substance of the plants, than any assimilation of it from that supplied in the free and gaseous form. In fact, he concluded that the source of the nitrogen of plants was, more probably, the nitrogenous compounds in the soil, and the small amount of ammonia which he demonstrated to exist in the atmosphere.

De Saussure further maintained the essentialness of the incombustible constituents of plants, and pointed out that they were derived from the soil. In his view, the fertile soil was the one which yielded liberally to the plants, nitrogenous compounds, and the incombustible or mineral constituents. He also called attention to the probability that the incombustible or mineral constituents of plants were the source of those found in the animals fed upon them.

Although there still remain to be solved, questions of vast scientific as well as practical interest regarding the conditions under which different plants take up their carbon, hydrogen, and oxygen, yet those who devote themselves to agricultural chemistry soon find that the explanation of the chemical phenomena of agricultural production awaits much more for a further elucidation of the sources, and modes of assimilation, of their *nitrogen*.

In 1837, Boussingault took up the subject of the sources of the nitrogen of plants, where De Saussure had left it more than thirty years before; being apparently led to see the importance of a further investigation of the question by a consideration of the

chemical statistics of certain agricultural practices on the large scale.

To the investigations and conclusions of Bouissingault, and others, reference will be made further on; but before doing so, or entering upon the consideration of our own experimental evidence regarding the assimilation of nitrogen by plants, attention will be called to a few prominent and striking facts established here, at Rothamsted, illustrative of the amounts of nitrogen yielded by different crops over a given area of land, and of the relation of these to certain measured, or known, sources of it.

I.—*Annual yield of Nitrogen per Acre in different Crops.*

The following summary table shows the average annual yield of nitrogen per acre, in the respective crops, when each was grown year after year on the same land, without manure, or with mineral, but without nitrogenous manure.

TABLE I.

Crop.	Seasons.	No. of years.	Nitrogen per acre per annum. lbs.
Wheat.....	1844—1859	16	24·4 without manure.
Barley.....	1852—1859	8	24·7 " "
Beans	1847—1858	12	47·8 " "
Meadow-Hay	1856—1862	7	39·7 " "
Turnips	1845—1852	8	42·0 with mineral manure.

In each case, the land was, in an agricultural sense, exhausted before the commencement of the experiment; that is to say, it would, in ordinary practice, have required manure before growing another crop.

It is seen that, without manure, the two Gramineous crops (wheat and barley) gave an average of about 24½lbs. nitrogen per acre per annum; the Leguminous crop (beans) nearly twice as much, or 47·8lbs, and the mixed herbage of the meadow-hay, comprising both Gramineous and Leguminous plants, 39·7lbs. Lastly, the turnips, with mineral, but without nitrogenous manure, gave 42lbs.

In reference to these results it should be stated, that the wheat as yet shows little, if any, but the barley with its more superficial range of roots, more obvious indication of progressive decline in the

annual yield ; and at present the meadow land shows no signs of diminution of produce. The yield of the Leguminous crop (beans), on the other hand, has depreciated very considerably, the average annual yield of nitrogen over the first six years being 70lbs., and that over the concluding six years only 26lbs. The turnips again, appeared to have greatly exhausted the immediately available nitrogen in the soil, within the range of collection of the barley plant ; for, on barley succeeding the eight mineral-manureo turnip crops, the produce was only about three-fourths as much as that obtained, in the same season, where barley was grown the second year in succession without manure, and only about three-fifths as much as where barley was grown as the second crop, of the second course, in a series of entirely unmanured four-course rotation-crops.

It should be further explained, that turnips, grown year after year without manure of any kind, yield, after a few years, only a few cwt. of produce per acre ; that purely Gramineous crops have given very little gain in the annual yield of nitrogen by the use of mineral without nitrogenous manures ; but that Leguminous crops, like the turnips just referred to, have given a considerably increased crop, and yield of nitrogen, under the influence of purely mineral manures.

In further illustration of the larger amount of nitrogen yielded over a given area in Leguminous than in Gramineous crops, the following remarkable results may be cited. Red clover was grown in three out of four consecutive years—the intermediate crop being wheat—all without manure :—

TABLE II.

Season.	Crop.	Nitrogen per acre. lbs.
1st year, 1849.	Clover	206·8
(2nd year, 1850	Wheat	45·2)
3rd year, 1851	Clover	29·3
4th year, 1852.	Clover	111·9
Average of the three years' clover.		116·0

All further attempts to grow clover, year after year, on this land, have, however, failed. Neither ammonia-salts, nor organic matters rich in carbon, nor mineral manures, nor mixtures of all,

have availed to restore the clover-yielding capabilities of the land. On the other hand, it should be particularly observed that, after taking 206·8lbs. of nitrogen from an acre in the clover crop of the first year, the wheat crop of the succeeding year was about double that obtained in the same season on a plot which had grown the crop for a series of years without manure, and equal to that obtained where during the same period farm-yard manure had been annually applied.

The following table shows the amount of nitrogen obtained per acre in wheat grown consecutively, in wheat alternated with beans, and in wheat alternated with fallow :—

TABLE III.
Period of Experiment ten years, 1850-9 inclusive.

			Nitrogen per acre. (lbs.)	
			Total.	Average annual.
Beans	} ten crops consecutively	Without manure	346·9	34·7
		With mineral manure	510·6	51·1
Wheat	} without manure	{ 10 crops consecutively	234·0	23·4
		{ 5 crops alternated with fallow	219·3	43·9 or 21·9
Wheat	} without manure	{ 5 crops alternated with beans	225·8	45·2 or 22·6
		{ 5 crops alternated with wheat	244·5	48·9 or 24·5
Wheat	} with mineral manure	{ 5 crops alternated with beans	207·0	41·4 or 20·7
		{ 5 crops alternated with wheat	227·2	45·4 or 22·7

The general result is seen to be, that pretty nearly the same amount of nitrogen was taken from a given area of land in wheat, in ten years, whether ten crops were grown consecutively, five in alternation with fallow, or five in alternation with beans. In fact, the crop of wheat was increased fully as much when it succeeded beans, which *carried off* a large amount of nitrogen, and of mineral matter also, as when it succeeded fallow, which *conserved and increased* the immediately available stores both of nitrogen and of mineral matter. It is also a remarkable fact, that *adding nitrogenous manures* is found to have much the same effect in increasing the produce of the wheat crop.

It will be seen by the illustrations next given, that the experimental results thus far adduced are consistent with those obtained

under circumstances more nearly allied to those of ordinary farm practice.

Taking the results of six separate courses of rotation, Bous-singault obtained an average of between one-third and one-half more nitrogen in the produce than had been supplied in manure. His largest yields of nitrogen were in the Leguminous crops; and the cereal crops were the larger, when they next succeeded the removal of the highly nitrogenous Leguminous crops.

In the experiments on this point at Rothamsted, the four-course rotation of turnips, barley, Leguminous crop (or fallow), and wheat, has been followed for twelve years—that is, through three separate courses,—*without manure*,* and the average annual yield of nitrogen per acre was 42·6lbs. The second and third courses gave, however, much less than the first, and hence, a smaller average per annum than that stated for the twelve years; the reduction being due to the falling off in the produce of the turnips, and of the Leguminous crop, whilst that of the barley and wheat has hitherto been fully maintained. The average yield of the twelve years is nearly twice as much as was obtained in wheat or barley, grown year after year, on the same land, without manure; and that of the last eight years even is considerably greater than the amount so obtained. The greatest yield of nitrogen was in the clover, which constituted the Leguminous crop of the first course; and the wheat-crops obtained after this clover, and after the beans in the second and third courses, were about double as much as the average where wheat has been grown succeeding wheat without manure, and about equal to the average obtained by farm-yard manure every year, or when wheat was grown after fallow, or after beans in the experiments last referred to.

The general result is, that, when grown year after year on the same land without manure, Gramineous crops gave about 24½lbs. of nitrogen per acre per annum, and Leguminous crops much more. Nevertheless, the Gramineous crop was nearly doubled when preceded by a Leguminous one; and it was also about doubled when preceded by fallow. Lastly, a series of entirely unmanured rotation-crops yielded considerably more nitrogen than the continuously grown unmanured Gramineous crops.

Further, the highly nitrogenous Leguminous crops were found

* The 16th crop, that is the last crop of the 4th course, is now growing, March, 1863.

to be comparatively little benefited by the direct application of nitrogenous manures in the form of ammonia-salts (though they are more so by nitrates), but considerably by mineral manures; whilst the cereals (wheat and barley), which yield a comparatively small amount of nitrogen, were much increased, both in amount of crop and yield of nitrogen per acre, by nitrogenous, but very little by mineral manures.

But, when the growth of crops (particularly the cereals) is increased by the direct application of nitrogenous manures, the increased yield of nitrogen over a series of years, bears a comparatively small proportion to the amount supplied in manure, as the following results will show :—

TABLE IV.

Crops.	Number of years.	Increased Nitrogen in produce for 100 in Manure.
Wheat	6	43·0
Barley	6	42·5
Meadow-Hay	7	44·8

Thus, over a period of six years, the increased yield of nitrogen in the wheat crop (compared with that without manure) amounted to 43, and in the barley crop to $42\frac{1}{2}$, per cent. of that supplied in manure; and it should be added, that the remainder does not appear to exist in the soil available for an immediately succeeding crop: for it has frequently been found that when an increased yield of nitrogen has been obtained in the season of application equal to about 40 per cent. of that supplied, the increase in the succeeding year, even with the aid of mineral manures, has only been equal to about one-tenth of the remainder.

In the case of the mixed herbage of the permanent meadow, the increased yield of nitrogen was slightly higher in proportion to that supplied than with the cereals. In Leguminous crops it is found to be even somewhat less; whilst root-crops (turnips) seem to gather up nitrogen in somewhat larger proportion to that supplied.

Let it be assumed that the increased yield of nitrogen approximately represents the proportion of that supplied by manure which is recovered in the crop, and the question arises what becomes of the unrecovered amount?

It may in part be drained away and lost. The nitrogenous

compounds may be transformed within the soil, and their nitrogen, in some form, evaporated; or they may for the most part remain in the soil in some fixed combination, to be yielded up only in the course of a long series of years. Ammonia, or nitrogen in some form, may be given off from the surface of the growing plant. Or there may be simply an unfavourable distribution of the supplied nitrogen within the soil for the immediate crop; and the Leguminous crop, alternating with the Gramineous one, may gather from a more extended range, and leave a residue, or allow the accumulation, of assimilable nitrogen within the range of collection of the crops which succeed it.

These considerations lead us to the next branch of our subject, namely—

II. *The actual or possible Sources of the Nitrogen of our Crops.*

The following actual or possible sources of the nitrogen of our crops, beyond that supplied in manure, may be enumerated:—

1. The nitrogen in certain constituent minerals of the soil, especially the ferruginous and aluminous, and certain nitrides.

2. The combined nitrogen annually coming down in the aqueous depositions of the atmosphere:—(a) as ammonia; (b) as nitric acid; (c) as organic corpuscles, &c.

3. The accumulation by the soil of combined nitrogen from the atmosphere:—(a) by surface absorption aided by moisture; (b) by the chemical action of certain mineral constituents of the soil; (c) by the chemical action of certain organic compounds in the soil.

4. The formation of ammonia, in the soil or the atmosphere, from free nitrogen (the so-formed ammonia either remaining as such, or being oxidated into nitric acid).

5. The oxidation of free nitrogen:—(a) by electric action; (b) with common oxygen, in contact with porous and alkaline substances; (c) under the influence of ozone, or nascent oxygen; (d) under that of evaporation, and combustion, as recently supposed by Schoenbein; ammonia at the same time being formed.

6. The direct absorption of combined nitrogen from the atmosphere by plants themselves.

7. The assimilation of nitrogen by plants.

A very few observations may be offered on each of these actual or possible sources of the nitrogen of vegetation.

(1.) The combined nitrogen of certain of the constituent minerals

from which soils are derived, cannot be considered an adequate source of the nitrogen annually carried off in the vegetable produce of the land.

(2.) The combined nitrogen coming down in the aqueous deposits of rain, hail, snow, mists, fog, and dew, part of which is the return from previously existing generations of plants or animals elsewhere, and part the product of new formation, does undoubtedly contribute materially to the annual yield of nitrogen in our crops; and being a source comparatively easily estimated, it has been the subject of a good deal of experimental investigation; more particularly by Boussingault, Barral, Way, and two of ourselves, and by others on a more limited scale.

The following table shows the amount of nitrogen coming down as ammonia and nitric acid in the total rain, hail, snow, and some of the minor deposits, during the years 1853, 1855, and 1856, here at Rothamsted, the nitric acid being in all cases determined by Mr. Way, and the ammonia in some cases by him, and in others by ourselves.

TABLE V.

	Nitrogen per acre, per annum, lbs.			
	1853.	1855.	1856.	Mean.
As ammonia.....	5·67	5·86	7·85	6·46
As nitric acid	(not determined.)	0·77	0·73	0·75
Total		6·63	8·58	7·21

It is seen that the available combined nitrogen so estimated, is computed to supply but a small proportion of that annually removed from an acre of land in different crops. Nor does it appear, from the results of Boussingault, that the amounts of combined nitrogen deposited by dew are such, that were correction made for the larger proportion so received by the soil, or the vegetation covering it, than was included in our collected and analysed aqueous deposits, any material modification in our approximate estimates would be required.

(3.) The amounts of combined nitrogen accumulated by the soil from the atmosphere, by surface absorption, or chemical action (whether due to restoration or to new formation), probably constitute a considerable proportion of that annually available for vegetation over a given area. Numerous investigations have been

undertaken, by ourselves and others, to determine the capacities for absorption of different soils, or constituents of soils; but, unfortunately, quantitative results obtained in the laboratory do not admit of direct and certain application to the estimation of the amount of combined nitrogen so fixed to a given depth, over a given area, within a given time.

(4 and 5.) The circumstances of the formation of ammonia, or of oxides of nitrogen, from gaseous, dissolved, or nascent nitrogen, are at present the subject of much conflicting statement; the various assumed actions being, as yet, by no means all clearly established qualitatively, and still less quantitatively. Moreover, here again, laboratory results would be difficult of application to the estimation of the probable amount of the nitrogen of vegetation due to such sources. To some of the actions in question further reference will, however, be made.

(6.) There is little of either qualitative or quantitative evidence in reference to the absorption of ammonia, or other compounds of nitrogen, from the air by plants themselves. Some vegetable physiologists, indeed, maintain that the leaves of living plants have not the function of absorption from the atmosphere, but only the converse one of evolution into it. Still, a few observations may be offered in connexion with the point in question.

Ripened cereal crops (wheat or barley) contain 1 part nitrogen to about 30 carbon, and Leguminous crops (beans, &c.) 1 part nitrogen to 15 or fewer of carbon. The atmosphere contains about 1 part carbon as carbonic acid to 10,000 of air; and it may be assumed, for illustration, to contain not more than 1 part nitrogen as ammonia to 12,000,000 of air;* or 1,200 times less nitrogen as ammonia than carbon as carbonic acid.

On these assumptions the ambient atmosphere contains a proportion of nitrogen as ammonia to carbon as carbonic acid, 40 times less than that of nitrogen to carbon in cereal produce, and 80 times (or more) less than that in Leguminous produce.

Hence, in point of *actual quantity merely*, the ammonia in the atmosphere would seem very inadequate to yield nitrogen in a degree corresponding to the yield of carbon by carbonic acid; nor does it appear, from observations hitherto recorded, that the nitrogen existing in the atmosphere, in combination with oxygen, would add much to that existing as ammonia.

* The amounts recorded by different observers range from 20·75 to 0·021 ammonia per million of air.

But water would absorb very much more nitrogen as ammonia, or dissolve very much more as carbonate or bicarbonate of ammonia, than it would of carbon as carbonic acid, under equal circumstances. In illustration, it may be mentioned that water at 60° F. (about 15·5 C.), would, at the normal pressure, absorb about 850 times as much nitrogen in the form of ammonia as carbon in carbonic acid; and, under equal circumstances, very many times more nitrogen as carbonate or bicarbonate of ammonia would be dissolved, than there would be of carbon as carbonic acid absorbed. There would appear to be, then, a compensating quality for the small actual and relative amount of nitrogen as ammonia in the atmosphere, in the greater absorbability, or solubility, of the compounds in which nitrogen exists, than of the carbonic acid in which the carbon is presented. How far the proportion of the nitrogen to the carbon available from the atmosphere in the combined form, may be really thus influenced under the actual conditions of vegetation, is a question, the numerous and intricate bearings of which, we do not profess here to enter upon.

One or two further observations may, however, be made as to the bearing of the actual facts of agricultural production on the point under consideration. Thus, Gramineous crops certainly depend very materially upon combined nitrogen *within the soil*, but are comparatively independent of carbonic acid yielded by manure in the soil; whilst Leguminous crops are at any rate much less benefited by the direct application of nitrogenous manures, from which it might, perhaps, seem that they were more able to avail themselves of nitrogen supplied in some way by the atmosphere, and possibly by the aid of their green parts. Even, should this be the case, it can hardly be to a greater *mere extent of surface above ground*, that the result is to be attributed. Thus, a bean and a wheat crop may yield equal amounts of dry matter per acre, but the bean-produce would contain 2 to 3 times more nitrogen, although approximate measurements show that the wheat plant offers a greater external superficies in relation to a given weight of dry substance, and, therefore, greater still, of course, to a given amount of nitrogen fixed. If, therefore, the bean can in some way assume more nitrogen from atmospheric sources than the wheat crop, the result must be due to *character and function*, rather than to mere extent of surface above ground. In connexion with this point it may be observed, more particularly with reference to the crops that are grown for their ripened seed, that

the Leguminous crops, however, generally maintain their green and succulent surface, in relation to a more extended period of the season of active growth, than do the Gramineous crops.

(7.) *Assimilation of free or uncombined Nitrogen by Plants.*—From the above brief review, it appears that those sources of combined nitrogen to plants which have as yet been quantitatively estimated, are inadequate to account for the amounts obtained in the annual produce of a given area of land beyond that attributable to previous manuring; whilst those which have not yet been quantitatively estimated (if even fully established qualitatively) offer many practical difficulties in the way of any such investigation of them as would afford results directly applicable to our present purpose. It seemed, therefore, desirable to endeavour to settle the question, whether or not that vast storehouse of nitrogen, the atmosphere, in which vegetation is seen to live and flourish, be of any measurable avail to the growing plant, so far as its *uncombined* nitrogen is concerned.

The settlement of this question would indicate the degree of importance to be attached to other points of inquiry. Were it found that plants generally, or some more than others, assimilated free nitrogen, much would be gained towards the explanation of certain chemical facts of agricultural production; it would be established that vegetation had the attribute of effecting chemical combinations with an element the most reluctant to associate itself with other bodies in obedience to laboratory processes, and apt to rid itself of connections once formed in the most violent manner; and we should be able more satisfactorily to account for the large actual amount of combined nitrogen existing and circulating in land and water, in animal and vegetable life, and in the atmosphere.

Moreover, the question of the assimilation of free nitrogen by plants, has, of late years, been submitted to an immense amount of research by numerous experimenters, and from the results very different conclusions have been arrived at. Hence, too, it seems desirable, before entering upon the discussion of our own experimental evidence on the point, to pass in review the methods, results, and conclusions of others, more particularly those of M. Boussingault who questions, and of M. George Ville who affirms, the assimilation of free nitrogen by plants; and as, so far as we are aware, the experiments of these other investigators have as yet been only very briefly noticed in English Scientific Journals,

we propose to give a somewhat fuller account of them than would otherwise have been necessary or appropriate.

III. *The researches of others, on the question of the assimilation of free Nitrogen by Plants, and some allied points.*

De Saussure, and his predecessors, sought to solve the question whether plants assimilate free or uncombined nitrogen, by determining the changes undergone in the composition of limited volumes of air by the vegetation of plants within them. Bous-singault pointed out that their methods were not fitted to attain the end in view. The general plan instituted by himself, and adopted with more or less modification in most subsequent re-searches, was :—

To set seeds or plants, the amount of nitrogen in which was estimated by the analysis of carefully-chosen similar specimens.

To employ soils and water containing either no combined nitrogen, or only known quantities of it.

To allow the access, either of free air (protecting the plants from rain and dust), of a current of air freed by washing from all combined nitrogen, or of a fixed and limited quantity of air, too small to be of any avail so far as its compounds of nitrogen were concerned. And finally—

To determine the amount of combined nitrogen in the plants produced, and in the soil, pot, &c., and, so, to provide the means of estimating the gain or loss of nitrogen during the course of the experiment.

M. Boussingault's Experiments.

The following table gives a summary of the results of M. Boussingault's experiments from 1837—1858, inclusive, on the question of the assimilation of free nitrogen by plants; also a brief reference to the conditions under which the results were obtained :—

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TABLE VI.

Summary of the results of M. Boussingault's Experiments.

Plants.	No. of seeds.	Period of growth; weeks.	Notes.	Grammes.				
				Seeds or Plants.	Produce, Dry.	Nitrogen.		
						In seeds (or plants).	In Products.	Gain or loss.
1837: Burnt soil; distilled water; free air; in closed summer-house.*								
Prefoil.....		8	Fine green, but meagre.	2.000	3.218	0.1100	0.1200	+ 0.0100
Prefoil.....		13	Much foliage, part withered.	2.000	6.288	0.1140	0.1560	+ 0.0420
Wheat.....	37	8	Weak and slender.	1.526	2.300	0.0430	0.0400	- 0.0030
Wheat.....	46	13	Lower leaves dead.	2.018	4.256	0.0570	0.0600	+ 0.0030
1838: Conditions as in 1837.†								
Peas.....	5	14	Flowers and seed.	1.211	4.821	0.0460	0.1010	+ 0.0550
Prefoil (plants).....	3	9	Flowered.	1.117	2.754	0.0330	0.0560	+ 0.0230
Oats (plants).....	4	7	Ripe seeds.	1.889	3.484	0.0590	0.0530	- 0.0060
1851 and '52: Washed and ignited pumice with ashes; distilled water; limited air; under glass shade, with carbonic acid.‡								
Haricot, 1851.....	1	8	Nascent flowers.	0.780	1.870	0.0349	0.0340	- 0.0009
Oats, 1851.....	10	8	Small pale leaves.	0.377	0.540	0.0078	0.0067	- 0.0011
Haricot, 1852.....	1	13	Good growth.	0.530	0.890	0.0210	0.0189	- 0.0021
Haricot, 1852.....	1	13	Open flowers.	0.618	1.130	0.0245	0.0226	- 0.0019
Oats, 1852.....	4	11	Ears shooting.	0.139	0.440	0.0031	0.0030	- 0.0001
1853: Prepared pumice, or burnt brick, with ashes; distilled water; limited air, in glass globe, with carbonic acid.‡								
White lupin.....	2	6	Vigorous growth.	0.825	1.820	0.0480	0.0483	+ 0.0003
Do. do.....	6	8	Very fine foliage.	2.202	6.730	0.1282	0.1246	- 0.0036
Do. do.....	2	7½	Very vigorous.	0.600	1.950	0.0349	0.0339	- 0.0010
Do. do.....	1	6	The finest obtained.	0.343	1.050	0.0200	0.0204	+ 0.0004
Do. do.....	2	7	Very active growth.	0.686	1.530	0.0399	0.0397	- 0.0002
Dwarf haricot.....	1	9	Nascent flowers.	0.792	2.350	0.0354	0.0360	+ 0.0006
Do. do.....	1	11	Open flowers.	0.665	2.800	0.0298	0.0277	- 0.0021
Garden cress.....	3	15	{ 3 grew, flowered and	0.008	0.650	0.0013	0.0013	0.0000
	10		{ seeded; 10 died.	0.026				
White lupins.....	2	19	{ 2 to grow, 8 as manure; }	0.627	5.760	0.1827	0.1697	- 0.0130
	8		{ vegetation magnificent. }	2.512				
1854: Prepared pumice with ashes; distilled water; current of washed air, and carbonic acid, in glazed case.§								
Lupin.....	1	9½	11 leaves, some blackish.	0.337	2.140	0.0196	0.0187	- 0.0009
Dwarf haricot.....	1	10	Open flowers.	0.720	2.000	0.0322	0.0325	+ 0.0003
Do. do.....	1	13½	Ripened seeds.	0.748	2.847	0.0335	0.0341	+ 0.0006
Do. do.....	1	15	1 small ripe seed.	0.755	2.240	0.0339	0.0329	- 0.0010
Do. do.....	2	14	3 ripe seeds.	1.510	5.150	0.0676	0.0666	- 0.0010
Lupin.....	1	7	{ 1 to grow, 1 as manure; }	0.810	1.730	0.0180	0.0334	- 0.0021
Do.....	1		{ very fine plant. }	0.300				
Cress.....	42	11	{ 30 grew, 12 as manure; }	0.100	0.533	0.0046	0.0052	+ 0.0006
			{ seeded. }					

* Ann. Ch. Phys., [2,] lxvii, (1838.)

† Ibid., lxix.

‡ Ann. Ch. Phys., [3,] xli, (1854.)

§ Ann. Ch. Phys., sér. [3,] xliii, (1855.)

|| Dry.

TABLE VI (*continued*).

Plants.	No. of seeds.	Period of growth; weeks.	Notes.	Grammes.				
				Seeds or Plants.	Produce, Dry.	Nitrogen.		
						In seeds (or plants).	In products.	Gain or loss.
1851, '52, '53, and '54: Prepared soil, or pumice with ashes; distilled water; free air, under glazed cage.*								
Haricot (dwarf), 1851 ...	1	15	{ Flowered and one im- perfect seed.	0.780	2.170	0.0349	0.0380	+ 0.0031
Haricot, 1852.....	1	13	Flowered and a pod.	0.537	2.110	0.0213	0.0238	+ 0.0025
Do. 1853.....	1	11	7 fine flowers.	0.655	2.720	0.0293	0.0270	- 0.0023
Haricot (dwarf), 1854 ...	1	10	4 fine flowers.	0.710	2.200	0.0318	0.0350	+ 0.0032
Lupin (white), 1853.....	1	13	Very fine, 11 leaves.	0.368	1.585	0.0214	0.0256	+ 0.0042
Do. 1854	1	12	Vigorous throughout.	0.341	1.960	0.0199	0.0229	+ 0.0030
Do. 1854 ...	2	8	8 leaves each.	0.630	2.180	0.0367	0.0387	+ 0.0020
Oats, 1852	4	15	Stems slender, ripe ears.	0.151	0.670	0.0031	0.0041	+ 0.0010
Wheat, 1853	5	15	{ 1 died; others 11 leaves each.	0.293	0.900	0.0064	0.0075	+ 0.0011
Garden cress, 1854	210	8	145 plants, each seeded.	0.500	2.225	0.0259	0.0272	+ 0.0013
1858: Nitrate of potassa as manure.†								
Helianthus.....{	2	13	Flowered.	0.116	1.168	0.0144‡	0.0130	- 0.0014
	2	13	Flowered.	0.116	2.120	0.0255‡	0.0245	- 0.0010

In Boussingault's experiments in 1837 and 1838, he grew his plants in burnt soil, watered them with distilled water, allowed free access of air, but kept them protected from rain and dust in a summer house. The tabulated results show that the dry substance of the produce was always much more than, and sometimes several fold that of the seeds sown, or plants planted, indicating, therefore, considerable growth, and consequent gain of carbon, hydrogen, and oxygen. They also show that in the case of the Leguminous plants, trefoil and peas, there was a gain of nitrogen varying from 10 to 55 milligrammes in the several experiments; whilst in that of the Gramineous ones, wheat and oats, in one instance there was a loss, and in another a gain of 3 milligrammes; and in another a loss of 6 milligrammes of nitrogen.

M. Boussingault's conclusions from the above results were in substance as follow:—That certain plants seem adapted to take up nitrogen in the atmosphere, but that it was a question, under what circumstances, and in what state, the nitrogen was fixed. He submitted—that the nitrogen might enter directly into the

* Ann. Ch. Phys., sér. [3] xliii. (1855).

† Compt. rend., xlvii. (1858).

‡ Nitrogen in seed and nitrate.

organism of the plant, provided its green parts were adapted to fix it; that it might be conveyed into the plant in the aërated water taken up by its roots; that there may exist in the atmosphere an infinitely small amount of ammoniacal vapour. He further suggested, that the gain of nitrogen beyond that supplied in manure, which he had observed in agricultural production, and which he thought came from the atmosphere, might be partly due to nitrate of ammonia produced by electric action and brought down by rain.

In 1851 and 1852, Boussingault confined his plants (in pots) in limited volumes of air under glass shades of about 35 litres' capacity, which rested in lutes of sulphuric acid, with tubes passing under for the supply of carbonic acid and water. Pumice-stone, coarsely powdered, washed, ignited, and cooled over sulphuric acid, served as soil, to which ash, from farm-yard manure and from seed of the kind to be sown, was added. In each year haricots and oats were grown, and the table shows that the haricots increased a good deal in dry substance, and in two out of three cases advanced to flowering, and some of the oats shot forth ears. There was, however, in every case, both with haricots (Leguminous) and oats (Graminaceous), a slight loss of nitrogen, varying from $\frac{1}{10}$ th of a milligramme to 2 milligrammes.

In 1853, the enclosing apparatus was a large globe or carboy of white glass, of 70—80 litres' capacity. At the bottom, prepared pumice stone (or burnt brick), and ashes, served as soil, which was watered with distilled water, and then the seeds were sown. The neck of the vessel was then closed with a cork, through a perforation in which a flask of carbonic acid was inverted, whose aperture, opening into the globe, was somewhat contracted. Finally, access of air was excluded by bandages of caoutchouc which rendered the whole apparatus air-tight.

In such apparatus, M. Boussingault made five separate experiments with white lupins, all of which grew more or less luxuriantly, and gained considerably in dry substance; but in no case was there as much as a milligramme of nitrogen gained; in three out of the five cases there was a slight loss, in one instance amounting to 0036 gramme. Two experiments were made with dwarf haricots, in both of which the gain of dry substance was considerable, and in one nascent, and in the other open flowers were developed; but in one case there was a gain of only 0006 gramme, and in the other a loss of 0021 gramme of nitrogen.

In an experiment with garden cress, in which three seeds grew and ten served as manure, abundant seed was produced, but there was neither gain nor loss of nitrogen. Lastly, in an experiment with white lupins, in which two seeds were sown to grow, and eight served as manure, the vegetation was very fine; but there was a loss of .013 gramme of nitrogen, attributed by Boussingault to free nitrogen given off in the decomposition of the organic matter supplied as manure. In such an apparatus, then, eight experiments were made with Leguminous plants, in most of which there was a loss rather than a gain of nitrogen.

To ascertain whether the limitation of growth in the closed vessels was due to the limitation in the amount of air, Boussingault sowed cress under otherwise equal conditions, but in a good soil, and the result was even more luxuriant growth than in a similar soil in the open air.

In 1854, Boussingault grew a number of pots of plants in a metal-framed glass case of 124 litres' capacity, through which, by means of an aspirator of 500 litres, he passed a current of air, washed first through sulphuric acid and then water. He also supplied carbonic acid, generally equal in amount to from two to three per cent. of the atmosphere of the apparatus; and distilled water was also supplied when needed. Prepared pumice, with ashes, served as soil.

Two pots of lupins, four of haricots, and one of cress, were grown in such an apparatus. The increase in dry substance was in all cases considerable; and in some cases flowers, and in others ripe seeds, were developed. In no case was there a gain of nitrogen amounting to one milligramme; and in four out of the seven there was a slight loss, amounting, in one instance, where a lupin had served as manure, to .0021 gramme.

Here, then, with a current of air, and with Leguminous plants, in six out of seven experiments, there was, upon the whole, a loss rather than a gain of nitrogen.

In 1851, 1852, 1853, and 1854, contemporaneously with the three series of experiments last described, Boussingault grew plants, so covered with a glazed cage as to exclude rain, or any material amount of dust, but allowing the free access of the external air. Haricots, lupins, oats, wheat, and cress, were so grown. The vegetation was in most cases vigorous. In several cases flowers, and in some seeds, were developed. In only one case was there less combined nitrogen determined in the total products, than was

estimated to be supplied. In the nine other experiments there was a gain of nitrogen indicated, varying from '001 to '0042 gramme, which Boussingault attributed to organic corpuscles and ammonia in the atmosphere.

Boussingault considered that, bearing in mind the circumstances of the above experiments, the gain was in no case sufficiently great to justify the conclusion that free nitrogen had been assimilated.

M. Boussingault made various collateral experiments to control or explain his results.

Various substances after being ignited were exposed to the air for two or three days, and then the combined nitrogen in them determined. A kilogramme, respectively, of sand gave 0.5, of powdered brick 0.5, of powdered bone-ash 0.84, and of wood-charcoal 2.9 milligrammes of ammonia.

To estimate the influence of organic corpuscles in the atmosphere, a pot of burnt sand, with ashes, the whole moistened with water, was so arranged under a glazed cage as to allow the free access of air, and, after exposure for two and a half months, small spots of cryptogamic vegetation were visible on the sand, but the whole yielded only 0.74 milligramme of nitrogen.

Ashes, if imperfectly burnt, were found in some cases to contain cyanides, in some ferrocyanides, and in others nitrogen in some other condition.

Boussingault considered that the larger gain of nitrogen indicated in his early experiments in free air (in 1837 and 1838) than in the later ones, was partly due to the comparatively defective methods of analysis at the early date, and partly to the distilled water then used containing some ammonia; for he had since learned, in his analysis of rain and other waters, that the distillate from water containing minute quantities of ammonia, did not come over free from it until about two-fifths of the whole had been drawn off.

To get increased vigour of growth, to avoid the loss of nitrogen apparently due to the evolution of free nitrogen during decomposition when organic matter was used as manure, and to determine whether the nitrogen of nitrates be assimilable by plants, Boussingault has latterly made some experiments in which nitrates were employed as manure.

In 1855, Boussingault grew two pots of helianthus, one without nitrogenous manure, and the other with a small known quan-

tity of nitrate of potassa supplied to the soil; also three pots of cress, one without nitrogenous manure, one with a manured soil, and one to the soil of which nitrate of soda was added. In the experiments with nitrogenous manure there was considerably increased assimilation of carbon, and much more nitrogen assimilated than was supplied by the seeds, but the excess was not equal to that supplied in the nitrate, where it was used.

Lastly, in 1858, Boussingault grew two separate pots of helianthus, using as soil sand and quartz, well washed and ignited, with the addition of nitrate of potass, in one case equal 0.0111, and in the other 0.0222 gramme nitrogen. In the first experiment there was 0.0014, and in the second 0.001 gramme less nitrogen found in the plant, soil, and pot, than was supplied in the seed and nitrate; and Boussingault supposed that nitrate had been decomposed in the soil by the organic matter of the debris of the seeds and the roots, and that nitrogen had been evolved.

From the results of these two experiments with nitrates, Boussingault concluded—that there was no assimilation of free nitrogen; that there was a loss of supplied nitrogen, either from the soil or by the plant; and that the amount of carbon assimilated bore a close relation to that of the nitrogen taken up by the plant.

The result is, then, that in Boussingault's experiments, extending over more than twenty years, and made under very varied conditions, neither with Leguminous plants nor others, did he find any assimilation of free nitrogen.

M. G. Ville's Experiments.

M. G. Ville, of Paris, commenced his investigations on the subject of the assimilation of free nitrogen by plants, in 1849.

He first determined the ammonia in the atmosphere by aspiring large quantities, over periods of several months, through acid. In this way he found the air of Paris to give a mean of 0.0237, and that of its suburbs 0.0211 parts by weight of ammonia to 1,000,000 parts by weight of air.

In M. Ville's experiments on the assimilation of nitrogen by plants, he used specially-made porous flower-pots, washed and ignited sand, sand and brick, or sand and charcoal, as soil, adding to it ashes. For the most part, several pots were enclosed in an iron-framed glazed case of 150 litres (or more) capacity, through

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which a current of air, washed or unwashed, was aspirated. Carbonic acid and distilled water were supplied as needed. In some cases ammoniacal gas was passed into the air of the apparatus. In others nitrates, or ammonia-salts, were supplied to the soil, and then the plants had free access of air, only shaded from rain and dust.

The following table summarises the chief results of M. Ville on the question of the assimilation of nitrogen by plants:—

TABLE VII.
Summary of M. G. Ville's Experiments.

Plants.	Period of growth; weeks.	Notes.	Dry substance. Grammes.		Nitrogen.—Grammes.			Nitrogen in products to 1 supplied.
			In seed (or plants).	In produce.	In seed; and air or manure.	In products	Gain or loss.	
1849: Current of unwashed air supplying 0·001 grm.: Nitrogen as Ammonia.*								
Cress	9		0·531	8·733	0·0260	0·1470	0·1210	5·6
Large Lupins ...	9		0·991	3·506	0·0640	0·0640	0·0000	1·0
Small Lupins ...	9		0·991	2·565	0·0640	0·0470	— 0·0170	0·7
					0·1530	0·2580	0·1030	1·7
1850: Current of unwashed air supplying 0·0017 grm.: Nitrogen as Ammonia.*								
Colza (plants) ...	17		0·599	53·761	0·0260	1·0700	1·0440	41·1
Wheat.....	17		0·682	2·807	0·0160	0·0310	0·0150	1·9
Rye	17		0·617	3·136	0·0130	0·0370	0·0240	2·8
Maize	17		1·488	4·503	0·0290	0·1280	0·0990	4·4
					0·0857	1·2660	1·1803	14·8
1851: Current of washed air.*								
Sunflower	13	95 rudimentary grains.	0·184	25·586	0·0050	0·1570	0·1520	31·4
Tobacco	13		0·084	22·436	0·0040	0·1750	0·1710	43·7
Tobacco	13		0·084	20·780	0·0040	0·1620	0·1580	40·5
1852: Current of washed air.*								
Autumn Colza ...	37	Flowered.	0·854	27·412	0·0480	0·2260	0·1780	4·7
Spring Wheat ...	16	47 complete grains.	1·194	12·917	0·0290	0·0650	0·0360	2·2
Sunflower	30	412 rudimentary grains.	0·588	64·090	0·0160	0·4080	0·3920	25·5
Summer Colza ...	15	much leaf.	3·723	60·408	0·1730	0·5950	0·4220	3·4
Summer Colza ...	10	much leaf.	1·833	64·786	0·1050	0·7010	0·5960	6·7
1854: Current of washed air (under superintendence of a Commission).†								
Cress	10	Did not thrive.	0·319‡	2·242	0·0099	0·0097	— 0·0002	1·0
Cress	10		0·124‡	6·021	0·0038	0·0530	0·0492	13·9
Cress	10		0·128‡	1·506	0·0039	0·0110	0·0071	2·8

* Recherches Expérimentales sur la Végétation, par M. Georges Ville. Paris, 1853.

† Compt. rend., 1855.

‡ Not dry.

TABLE VII (*continued*).

Plants.	Period of growth; weeks.	Notes.	Dry substance. Grammes.		Nitrogen.—Grammes.			Nitrogen in products to 1 supplied.
			In seed (or plants).	In produce.	In seed; and air or manure.	In products.	Gain or loss.	
1854: Current of washed air (closed, under the superintendence of a Commission).*								
Cress	7		0.206‡	3 599	0.0063	0.0350	0.0287	5.6
1855 and 1856: In free air; with 0.5 grm. : Nitre = 0.069 Nitrogen.†								
Colza	7		0.027	5.450	0.0700	0.0700‡	0.0000	1.0
Colza	7		0.027	5.140	0.0700	0.0660‡	— 0.0040	0.9
Colza	10		0.027	5.020	0.0700	0.0680‡	— 0.0020	1.0
1855 and 1856: In free air; with 1 grm. Nitre = 0.138 Nitrogen.†								
Colza	9		0.031	7.750	0.1400	0.1970‡	0.0570	1.41
Colza	12		0.031	15.300	0.1400	0.3740‡	0.2340	2.67
Colza	17		0.031	10.770	0.1400	0.2160‡	0.0760	1.54
Colza	18		0.031	22.230	0.1400	0.2500‡	0.1100	1.79
1856: In free air; with 0.792 grm.: Nitre = 0.110 Nitrogen.†								
Wheat.....	24			26.900	0.1260	0.2180‡	0.0920	1.7
Wheat.....	24			26.520	0.1260	0.2240‡	0.0980	1.8
1855: In free air; with 1.72 grm.: Nitre = 0.238 Nitrogen.†								
Wheat.....	26	Gave 84 grains.		37.370	0.2590	0.3080‡	0.0880	1.2
1856: In free air; with 1.765 grm.: Nitre = 0.244 Nitrogen.†								
Wheat.....	22	Flowered		26.87	0.2650	0.2170‡	— 0.0040	0.8
Wheat.....	29	Gave 119 grains		41.56	0.2690	0.3500‡	+ 0.1320	1.5

In 1849, M. Ville placed three pots of plants in his apparatus, one of cress, one of large lupins, and one of small lupins; sand was used as soil; and the aspirated air was unwashed. The cress plants gave more than sixteen-fold the dry substance of the seed sown; and about five and a half fold the nitrogen in the seed. The other plants did not gain nitrogen. The total nitrogen in the seed of the three pots was estimated at 0.154 gramme, that in the ammonia of the unwashed air 0.001, and that in the total products was 0.285, showing a gain in the three experiments

* Compt. rend., 1855.

† Recherches Expérimentales sur la Végétation, 1857.

‡ In plants only.

§ Not dry.

of 0.103 gramme, or 1.7 fold that supplied. M. Ville concluded that the cress had assimilated a considerable quantity of free nitrogen.

In 1850 four pots, one of colza, one of wheat, one of rye, and one of maize, were enclosed in the apparatus; and the conditions were the same as in 1849, excepting that the experiment with the colza commenced with young plants instead of seed. The produced colzas gave 40—50 fold the dry substance of the young plants; and the nitrogen also increased more than forty-fold. The other plants also gained, but in less amount and proportion. The total combined nitrogen in the contents of the apparatus was nearly fifteen times that supplied, 1.18 gramme being gained. The conclusion was that a large quantity of free nitrogen had been assimilated.

In 1851 the plants were supplied with a current of air washed free from ammonia. One pot of sun-flower, and two of tobacco (the latter starting from young plants), were grown together in the apparatus, with the conditions otherwise as before. The sun-flowers gave 95 rudimentary grains, but the tobaccos did not flower. The three experiments together gave nearly two hundred fold the original dry matter, and nearly forty fold the original nitrogen, the actual gain in the apparatus being 0.481 gramme.

In 1852 the conditions were as in 1851, and the plants grown were autumn colzas (from plants), spring wheat (from seed), sun-flower (from seed), and summer colzas (from plants). All gave a considerable increase of dry matter; and the total combined nitrogen gained in the apparatus was 1.624 gramme, or 5.3 times as much as was supplied in the original plants and seed.

In each of the years 1850, 1851, and 1852, M. Ville had a duplicate apparatus, with plants similar to those in the experiments above described, to which, however, he supplied ammonia in the atmosphere of the apparatus. These duplicate plants increased much more, both in dry substance and in nitrogen, than the others; but the gain of nitrogen was in no case equal to that supplied as ammonia, and hence, the results have not a direct bearing upon the question of the assimilation of free or uncombined nitrogen.

Up to 1853, inclusive, M. Boussingault had experimented either in free air, with protection from rain and dust, or in fixed and limited air, and, considering the conditions, in neither case was it concluded that free nitrogen had been assimilated.

M. G. Ville's experiments up to the same period, had, on the other hand, indicated an enormous gain of nitrogen. Results so strikingly contradictory, naturally excited great attention; but the fact that M. Boussingault's plants had not been supplied with a *current* of air, and some other circumstances, were alleged to account for the difference in result. In 1854, M. Boussingault commenced experiments with a current of air, the results of which have already been given, and a Commission of members of the Academy of Sciences of France, comprising MM. Dumas, Regnault, Payen, Decaisne, Peligot, and Chevreul, was appointed to superintend a new set of experiments by M. Ville.

M. Ville's experiments under the Commission were conducted at the Muséum d'Histoire Naturelle, Jardin des Plantes, Paris; M. Cloez was appointed to assist him, and M. Chevreul reported on behalf of the Commission in 1855. In one apparatus connected with the aspirator three pots of cress were placed, and in another smaller one (which was not opened during the experiment, as the other frequently was,) one pot of cress was grown. In one pot, in the set of three experiments, there was no gain; in another a gain of 0·0492; and in the third, a gain of 0·0071 gramme of nitrogen. In the permanently closed apparatus there was a gain of 0·0287 gramme nitrogen.

Unfortunately, an element of uncertainty attached to these experiments under the Commission. A quantity of distilled water from the bulk used for watering the plants, was in course of evaporation with oxalic acid under the superintendence of M. Cloez, when he was called away for some days; and when M. Peligot determined the ammonia in the acid residue, and also in that of the water removed from the cases, he found such an excess of ammonia in the water before being used over that in the water removed from the larger case, as more than covered the increase of nitrogen in the experiments. But when new portions of the original water were examined, a different result was obtained; and M. Cloez found that the previous evaporation had, in his absence, gone on by the side of ammoniacal emanations. However, the result with the single pot, in the small apparatus, showed a considerable gain of nitrogen, even supposing the first analysis of the supplied water to be correct.

From the result of the whole inquiry, the Commission announced the following conclusion:—

That the experiment made at the Muséum d'Histoire Naturelle,

by M. Ville, is consistent with the conclusions which he has drawn from his previous labours.

In 1855 and 1856, M. G. Ville conducted experiments in which the plants were allowed to grow in free air, only shaded from rain, and of which the special conditions were, that nitrate of potass in smaller or in larger quantity, or nitrate of potassa and different ammonia-salts, in equivalent quantities as to nitrogen contents, were employed. Artificial soils, with ashes of plants, and distilled water, being used as before.

With a view to these experiments, he devised a method for estimating minute quantities of nitric acid, which was favourably reported upon by M. Pelouze, on behalf of a Commission, composed of MM. Balard, Peligot, and himself.

In 1855 two pots, and in 1856 one pot, of colzas were grown, to each of which 0.5 gramme of nitre was supplied. The dry substance increased about two hundred fold; and analysis showed that the total produce contained almost exactly the same amount of nitrogen as that supplied in the seed and the nitrate. In each of the same years two pots of colzas were grown, to each of which 1 gramme of nitre was supplied. The dry matter of the produce was several hundred times as much as that of the seed sown; and it contained considerably more nitrogen than had been supplied in the seed and nitrate. M. Ville concluded that when, by the larger supply of nitrate, the growth had been extended, free nitrogen was assimilated.

In 1855, a pot of wheat was grown manured with 1.72 gramme of nitrate of potass. Eighty-four grains were produced; and there was more nitrogen in the produce alone, than in the seed and nitrate, and much more when the residue in the soil was taken into account. In 1856, two pots of wheat were sown to each of which 1.765 gramme of nitre was added. The plants of one pot were taken up at the time of flowering, and found to contain almost as much nitrogen as was supplied in the seed and nitrate. Those in the other were left as seed, and gave 119 grains; and the nitrogen in the produce, exclusive of the residue in the soil and pot, was much more than that supplied. Also, in 1856, two pots of wheat were sown without nitrate, and two with 0.792 gramme each. The actual gain of nitrogen was the greater where the nitrate was employed, but the proportion gained, to that supplied, was greater where no nitrate was supplied.

In 1856, experiments were also made, comparing the effects of

nitrates and ammonia-salts, from the results of which it was concluded, that a given amount of nitrogen was more efficacious in the form of nitrate, than in that of ammonia-salts.

In regard to the experiments made in 1855 and 1856, in free air, with nitrates or ammonia-salts supplied, M. Ville remarks that the point at which the supplied nitrogen becomes exhausted is indicated by a lightening of the colour of the leaves, and that it is then that the plants begin to assimilate free nitrogen, to secure which it is necessary that the supply of nitrogen, and the vigour of growth, should reach a certain point, but that the supply should not exceed a certain limit. Further, that the gain of nitrogen in the experiments in question was so great, and the amount before shown to be available from atmospheric ammonia was so small, that the influence of that source may be entirely overlooked. He enumerates the following conclusions:—

1. By means of nitre we may prove, without the aid of an enclosing apparatus, that plants absorb and assimilate the gaseous nitrogen of the atmosphere.

2. Nitre acts by its nitrogen. It is absorbed in the state of nitre.

3. In relation to the amount of nitrogen, nitre is more active than ammonia-salts.

M. Ville also made various collateral experiments.*

Air was passed through an otherwise closed apparatus, in which was placed a vessel containing calcined sand, or calcined sand and decomposing organic matter, but in no case was nitric acid formed; hence, nitrification was not the source of the nitrogen gained by the experimental plants.

Given amounts of organic matter, lupins, gelatine, &c., were mixed with calcined sand, and exposed in an apparatus to a current of air which carried the gaseous products through acid. The determination of the nitrogen remaining in the matrix, and of that absorbed as ammonia by the acid, showed a loss of nitrogen given off in the free state. Somewhat similar experiments were made, in which seeds of wheat were sown in the sand, decomposing organic matter serving as manure. When the growth was allowed to continue long enough, the products contained more nitrogen than the seeds sown and the organic matter; in these cases it could not be said that the nitrogen not received by the plants as

* *Recherches Expérimentales sur la Végétation*, 1857.

ammonia had been taken up as nascent nitrogen evolved in the decomposition.

The conclusions from the collateral experiments were as follow :—

1. Organic matters in decomposition lose a part of their nitrogen as ammonia, and a part as nitrogen gas.

2. Vegetation does not interfere with the progress of this decomposition.

3. Plants cultivated in a manured soil, give more nitrogen in their produce than the manure yields as ammonia.

4. The excess of nitrogen in the produce has been absorbed as free gaseous nitrogen.

In regard to the explanation of the assimilation of free nitrogen by plants, M. Ville calls attention to the fact, that nascent hydrogen is said to give ammonia, and nascent oxygen nitric acid, with free nitrogen, and he asks,—why should not the nitrogen in the juices of the plant combine with the nascent carbon and oxygen in the leaves? He further refers to the supposition of M. De Luca, that the nitrogen of the air combines with the nascent oxygen given off by the leaves of plants, and to the fact that the juice of some plants (mushrooms) has been observed to ozonize the oxygen of the air, and asks—Is it not probable, then, that the nitrogen dissolved in the juices will submit to the action of the ozonized oxygen with which it is mixed, when we bear in mind that the juices contain alkalies, and penetrate tissues the porosity of which exceeds that of spongy platinum?

Results and conclusions so astonishingly conflicting as those of M. Boussingault and M. G. Ville, have naturally incited others, either to investigate anew, or to seek in the conditions provided in their experiments, for some explanation of the discordance. A brief notice of the labours, or opinions, of these other experimenters or arbitrators, may be here subjoined.

In 1851, M. Mène* made three sets of experiments :—

1. Wheat and peas were, respectively, grown in powdered-glass, in free air, and watered with pure water. The wheat gained nitrogen equal to one-fourth of that in the seed sown, and the carbon, hydrogen, and oxygen, were doubled. The peas doubled the carbon, oxygen, and hydrogen, and trebled the nitrogen of the seed.

2. Lentils, peas, haricots, beans, wheat, rye, and oats, were

* Compt. rend. xxxii.

grown in a sterile matrix under a bell-glass, and supplied with an atmosphere of known composition, and with acetate of ammonia in the soil. The plants gained nitrogen, and the soil lost ammonia, but the nitrogen of the air was not perceptibly affected.

3. This series was similar to the second, but with the nitrogen of the air replaced by hydrogen. The plants flourished, and took up some of the acetate of ammonia.

M. Mène concluded that plants do not appropriate the free nitrogen of the air.

M. Roy, writing in 1854,* supposed that carbonate of ammonia was the chief source of nitrogen to vegetation; that Leguminous plants appropriated it from the atmosphere by their leaves; that Gramineous plants only took it up in solution by their spongioles; and that free nitrogen was not fixed by the leaves of plants, but when dissolved in water, and taken up by their roots, it could be assimilated. He concluded, that in M. Boussingault's experiments in limited air, there would be but little passage of solution of nitrogen by the roots, and evaporation of water from the leaves, and hence, not the necessary conditions for the assimilation of free nitrogen; but that with M. Ville's rapid current of air it would be otherwise.

In 1850, MM. Cloez and Gratiolet published the results of some experiments with water-plants, from which they concluded, that in the vegetation of such plants, nitrogen is given off from their nitrogenous constituents; that there must be restoration either from free or uncombined nitrogen; and that as their experiments showed ammonia-salts to be injurious to the plants, they probably take up free nitrogen dissolved in water.

In 1855, M. Cloez † published the results of some experiments on nitrification, in which he passed washed air for several months through twenty different combinations of porous, earthy, and alkaline matters. He found nitrates to be formed in notable quantity in calcined brick, or pumice, impregnated with alkaline or earthy carbonates, also in uncalcined brick similarly impregnated; but only traces in chalk, marl, a mixture of kaolin and precipitated carbonate of lime, &c. He considered, therefore, that the porosity of the pots and brick-fragments, the alkalinity of the ashes, the moisture, and the current of air, in M. Ville's experiments, provided the conditions for the formation of nitric acid. He asks, can such formation take place in limited air?

* Compt. rend., xxxix.

† Compt. rend., xli.

M. De Luca * aspirated a large quantity of air in the neighbourhood of vegetation, through carded cotton, and then through sulphuric acid to wash it. The washed air then passed over potassium and through a dilute solution of pure potash, when nitrate was formed; but when air in the midst of habitations was operated upon in a similar way the formation of nitric acid was not observed. M. De Luca had found that the oxygen given off by plants in sun-light was in many cases ozonous, and supposed that by its means the nitrogen of the air may be converted into nitric acid, and that thus the nitrogen of the air may be rendered available for assimilation by plants, under the influence of vegetation itself.

In 1855, M. Harting † published some observations, and the results of some experiments, on the question of the assimilation of nitrogen by plants. He attributed a formation of ammonia from the decomposing débris of seeds, &c., and the free nitrogen of the air, in M. Ville's experiments; and he also supposed that nitric acid might be formed by the oxidation of the atmospheric nitrogen. The increase of nitrogen in M. Ville's plants, and of ammonia in the water of the enclosing apparatus, was taken as a proof of such formation of ammonia. He made two sets of experiments, in one of which the plants grew in a limited volume of air, and in the other in a current of air washed free from ammonia, both being arranged with a view to avoid the formation of ammonia. However, the produced plants yielded no more dry matter than was contained in the seeds, and M. Harting considered, therefore, that the determination of the nitrogen was superfluous. The growth evidently stopped when the supplies of the seeds were exhausted. His general conclusions were as follow:—

1. Plants absorb salts of ammonia, and nitrates, by their roots.
2. The nitrogen of the air contributes to the formation of ammonia, and nitrates, in the soil.
3. It is not proved that nitrogen serves directly for the nutrition of plants.

In 1852 and 1853, M. H. M. Chlebodarow made some experiments at Dorpat, under the direction of M. Petzholdt, who reported upon the results. ‡ M. Petzholdt assumed that if plants can appropriate the free nitrogen of the air, they will not need

* Compt. rend., 1856.

† Compt. rend., xl. (1855.)

‡ J. pr. Chem., lxx.

ammonia, and that if they take nitrogen from ammonia the supply of it will increase growth. The experiments were made upon barley. In 1852 an ignited yellow sand was used as soil. To one set of plants no ammonia was supplied, to a second carbonate of ammonia was provided in the soil, and to a third it was supplied in the air. The crops with a supply of ammonia gave three times as much produce, and much more nitrogen, than those without it. In 1853, six sets of experiments with barley were made, the soils consisting of an artificial mixture of clay, sand, and felspar decomposed by heating with lime. To one set of three pots, no manure was added; to the second, a small quantity of bone-ash, decomposed by sulphuric acid; and to the third, a larger quantity of the same phosphatic mixture. The three other sets were arranged like the above, and had, in addition, ammonia supplied to the atmosphere in which the plants grew; the experiments, without ammonia, being made in free air, and those with it in an enclosing apparatus. The nitrogen of the crops was very much increased by the aid of the phosphatic manure, and still further by the addition of the ammonia to the atmosphere of the plants. M. Petzholdt considered it difficult to account for the fact of M. Boussingault getting little or no increase of nitrogen in free air, which must have supplied some ammonia, even though rain and dew were excluded.

The results or conclusions of these several arbitrators are seen to be nearly as conflicting as those of M. Boussingault and M. G. Ville themselves, nor are we able to discover, either in the differences of plan, as described by M. Boussingault and M. G. Ville themselves, or in the results and explanations of other experimenters, a satisfactory solution of the difference of result arrived at.

From the chemical characters of nitrogen itself, and from what is known of the chemistry of vegetation in other respects, it would be concluded that plants would not assimilate nitrogen offered to them in the free state. On the other hand, the total amount of actually existing combined nitrogen is very large, and there is an amount of nitrogen periodically available for the vegetation of a given area of land, the source of a considerable proportion of which is, to say the least, not yet quantitatively explained; and, upon the decision finally come to in regard to the question whether or not the assimilation of free nitrogen

by plants, may account for all, or a part of the otherwise unexplained amount, must materially depend the degree of importance to be attached to the further investigation of the other actual or possible sources of nitrogen to plants which have been briefly noticed. It seemed desirable, therefore, that the subject should be submitted to further inquiry, and we now proceed to give a condensed account of our own experimental evidence on the point.

IV.—*Experimental results obtained at Rothamsted in 1857, 1858, and 1859, on the question of the Assimilation of Free Nitrogen by Plants, and some Allied Points.*

In the treatment of this part of our subject, it is proposed to consider—

1. The conditions required, and plan adopted, in the direct experiments on the question of the assimilation of free nitrogen by plants: embracing those which relate to the requirements and preparation of the soil or matrix, and of the nutriment to be supplied to the plants, the selection of plants and seeds, the atmosphere to be provided, the description of apparatus adopted, and the mode of using it (which should be so arranged as to include all that is necessary for the healthy and vigorous growth of the plants, excepting, in some instances, a full supply of combined nitrogen), and lastly, the question of the analytical methods adopted, and of their competency to serve the purposes of the enquiry.

2. A number of collateral questions having a bearing upon the points at issue: including those of the possible influence of ozone on the supply of combined nitrogen to growing plants, of the action of ozonised air on decomposing organic matter, and porous and alkaline substances, of the evolution of free nitrogen in the decomposition of nitrogenous organic matter, and of the mutual relations of gaseous nitrogen and the nascent hydrogen evolved during the decomposition of organic matter.

3. The results of the direct experiments themselves on the question of the assimilation of free nitrogen by plants.

Conditions required, and plan adopted, in Experiments on the question of the Assimilation of Free Nitrogen by Plants.

So complicated is the constitution of ordinary soils, and so intimately are the nitrogenous compounds existing within them associated with other matters, that it is impossible either to estimate their

nitrogen with sufficient accuracy for our present purpose, or to extract it from the soil without entirely destroying the other conditions of vegetable growth ; nor could a natural soil be so imitated by artificial means, as to include all its conditions excepting a supply of combined nitrogen. There exists, however, abundant evidence to show that many of the complicated conditions of an ordinary soil may be entirely dispensed with, so as to bring the examination within our means of investigation, and yet, to retain all the conditions of healthy growth. The experiments of others to which attention has been directed, are sufficiently conclusive on this point, and the results now to be recorded bear similar testimony.

In the experiments of the first year, 1857, two kinds of soil, or matrix, were used ; one prepared from an ordinary soil, and the other from volcanic pumice. The latter substance was employed with a view to eliminate certain supposed sources of error which the prepared soil might introduce ; but results, to which reference will be made, showed the precaution to be unnecessary, and, in 1858, only prepared soil was used.

A somewhat clayey soil was selected ; and, after the large stones had been removed, and the clayey lumps powdered, it was ignited in a large cast-iron muffle, through which a constant current of air passed, until, on removing a portion, and cooling it, it exhibited the red colour due to peroxide of iron, and showed no traces of coaly matter. The mass was then thrown into a large volume of distilled water, which was rendered strongly alkaline by the caustic lime present. After stirring, and being allowed to settle, the fluid was decanted, then fresh water added, and the operation repeated frequently during eight or ten days, until all the soluble matter was removed. The residue was then dried and retained for final ignition before use.

The ferruginous and aluminous character of this soil-matrix pointed to the possibility of its acting as a porous body in promoting the formation of nitrogenous compounds, or absorbing and retaining the ammonia supplied to the plants, or that which might be formed from the nitrogenous matter of the seed. In order to ascertain whether any formation of nitrogenous compounds in the manner here supposed occurred, a pot of soil, prepared as for an experiment with a plant, was submitted to the same conditions of air, temperature, moisture, &c., as the pots containing the experimental plants, but no accumulation of combined nitrogen took place.

When volcanic pumice was used, it was powdered until the greater portion was quite fine, and the largest pieces were about the size of peas. The powder was then subjected to long washing in the same manner as the ignited soil, and lastly, dried ready for final ignition before being used.

The necessary mineral constituents were in most cases supplied in the form of the ash of the plant of the description to be grown, or, if this were not practicable, of that of some closely allied kind. In some instances the ash was obtained by burning a quantity of the entire plant when in seed; in others, the seed and the rest of the plant were burnt separately, and a mixture of the two ashes made in the proper proportions. Strongly alkaline ashes (of Leguminous plants) were sometimes saturated with sulphuric acid, and the mixture ignited. The ashes were prepared in large shallow platinum dishes, heated in a current of air in a cast-iron muffle, and the burning was continued until all coaly matter disappeared. Examination failed to detect combined nitrogen in any of the ashes so prepared. A consideration of the chemical constitution of soils suggested a proportion of 0·8 to 1·0 per cent. of ash, and this was the quantity added to the matrices in 1857, but in 1858 only about half as much was employed.

In some cases weak solutions containing phosphoric acid or sulphuric acid were used as well as ash.

The strength of a dilute solution of phosphoric acid was determined by means of a titrated alkali-solution, and it was then neutralised by carbonate of soda. Each pipette measure of the solution given to the plants supplied about 0·01 gramme phosphate of soda. It was only employed in the experiments of 1858.

The sulphuric acid solution, which was also only employed in 1858, was prepared by determining the strength of some very dilute pure acid, in the same manner as that of the phosphoric acid. It was then so far reduced that the pipette measure by which it was applied to the plants contained exactly as much sulphuric acid as the pipette of sulphate of ammonia solution then in use, which corresponded to 0·004 gramme nitrogen.

When ammonia was supplied to the plants, it was given in the form of a weak solution of the sulphate, which was prepared as follows:—Ordinary ammonia-water was distilled from a flask and the vapour condensed in pure distilled water. The strength of the solution was determined by the volumetric method against that of dilute sulphuric acid of known strength, the preparation

of which is described further on. A given volume of the ammonia-liquid was then neutralised by pure dilute sulphuric acid, of which the measure added was determined, and the strength of the sulphate-solution calculated accordingly. It was intended that each cubic centimetre should contain about one-tenth of a milligramme of combined nitrogen, and generally 4 to 6 milligrammes were supplied at once.

The value of each of the above solutions was determined by analysis to insure that its composition was such as was supposed.

The plants were watered with distilled water, in the preparation of which the first two-fifths of the distillate from ordinary water were allowed to escape, and the next two-fifths were collected for further treatment. This water retained traces of ammonia. It was mixed with a little phosphoric acid free from nitric acid, and then re-distilled from a copper vessel, to which a glass condenser was attached.

All parts of the apparatus, the presence of ammonia in which could possibly affect the result, were, after thorough washing both with ordinary and common distilled water, finally rinsed with this pure double-distilled water just before being used.

In the experiments of 1857, common flower-pots were used, and small common white-glazed plates were employed as pans. For the experiments of 1858, observation suggested the kind of pot and pan represented in figs 1, 2, and 3, plate I. The pot was made of the same material as ordinary flower pots, but as light as possible, and it was somewhat less baked. The height and diameter at the top were each five inches, and the diameter at the bottom was four inches. The bottom, and the sides to a distance of one inch from the bottom, were perforated with numerous holes of nearly one-fourth of an inch diameter as shown in figs. 1 and 2. The pan, represented in fig. 3 with the pot placed in it, is made of hard baked and well-glazed stoneware. It is 1.5 inch deep and 5.2 inches in diameter at the bottom. At the top it is curved inwards (A, B, fig. 3), so as to adapt its upper rim to the sides of the pot. These arrangements reduced the surface for evaporation from a given volume of soil, and facilitated the exit of the roots, and the access of air. The pan affords room for an abundance of water, in which the roots develop luxuriantly, and are protected from the direct action of sun-light. Thus, a minimum of evaporation from other sources, and a drier atmosphere, being maintained, evaporation through the plant itself is favoured; and hence

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

the conditions are provided for a constant supply to the plant of all the mineral and gaseous substances in solution in the fluid of the soil and pan.

In preparation for an experiment, the soil, the ash, and the pot, were simultaneously heated to redness, and the soil and ash, whilst red-hot, were mixed together in the red-hot pot, which was placed upon a red-hot brick, over sulphuric acid, the whole being then covered with a large glass shade, and left to cool. In 1857, from $2\frac{1}{2}$ to 3lbs. of ignited soil were put into each pot; but, in 1858, the lower part of the pot was first filled, to the depth of about one inch, with very coarsely broken up red-hot flint. The greater portion of the ash was mixed with the lower layers of soil, but some was distributed through the whole of it. After cooling sufficiently, the shade was removed, and about 500 cub. centims. of pure distilled water were added to the soil of each pot; and, after ten to twenty hours more, the seeds were put in.

In all the experiments recorded, the plants were grown directly from-seeds, sown in soils prepared as above described. A quantity of seed of very good quality being procured, the largest and smallest, or any looking unhealthy, were picked out. Given numbers of the remainder were then weighed, and the average weight per seed was calculated. A few, weighing as nearly as possible the mean weight, were then selected for planting, and a number of others, of equal weight and character, were reserved for the determination of their nitrogen, as a means of estimating the quantity supplied in the seed sown.

The quantity of ammonia in the atmosphere is so small, that, had it been intended to grow the experimental plants in limited volumes without change, the amount of combined nitrogen available to the plants by its means might have been entirely disregarded; but as, for various reasons, it was decided that the plants should grow in an enclosing apparatus through which a current of air passed, it was freed from ammonia, and solids mechanically suspended, by washing in the manner presently to be described, before being admitted into the apparatus.

Owing to the small proportion of carbonic acid in the atmosphere, and to the fact, that a part of it would be absorbed in the washing process just referred to, it was necessary to give a supply of it to the plants artificially. This was obtained by the action of chlorhydric acid upon fragments of marble, and the arrangements for its supply will be described presently, with the rest of the

apparatus. Boussingault found* that the air surrounding a plant might, consistently with healthy growth, contain 8 per cent. of carbonic acid, whilst the normal atmosphere, in which vegetation flourishes, contains only 0.04 per cent. Calculation showed that a minimum of 0.2 per cent of carbonic acid in the air of the enclosing apparatus would supply very much more than was required by the plants: and from the observations of Boussingault it was concluded that 4 per cent. might be adopted as the maximum. Further, our own experiments on the nature of the gas in plants show (as will be seen further on) that the changes in the proportion of carbonic acid in the air of the cells and intercellular passages, and in the fluids of the stem, are much greater and more rapid than those which could take place in the atmosphere of our experimental plants. Moreover, plants derive much of their carbonic acid from aqueous solution absorbed by the roots, and most probably the remainder is taken up by the fluids of the plant before influencing its growth. These absorptions can take place but slowly, so that rapid variations in the proportion of carbonic acid in the atmosphere of the plant, will be accompanied by much less variation within it. It was considered probable, therefore, that there would be no danger in so supplying carbonic acid to the atmosphere of the plants, as to reach the proposed maximum proportion in a short time, and then, by the passage of the air, bring it gradually down to the minimum.

In practice, a little more chlorhydric acid was added at one time to the marble than was necessary to provide 4 per cent. of carbonic acid in the atmosphere of the plants, and the passage of the air was commenced simultaneously. Repeated analyses of the air in the enclosing apparatus showed that the limits of 4 per cent. as the maximum, and 0.2 per cent. as the minimum, were not passed when operating in this way.

The description of apparatus used for each separate experiment in 1858 (and subsequently), to enclose the plants, and to supply them with air, water, carbonic acid, &c., is represented in fig. 1, plate II; and that used in 1857 only differed in some few points, to the chief of which reference will be made.

A, represents a large stoneware Woulfe's bottle, 18 inches in diameter, and 24 inches high. *a* represents the cross section of a leaden pipe $1\frac{1}{2}$ inch diameter (which is connected with a reservoir

* *Mémoires de Chimie Agricole et de Physiologie*, 1854, p. 441.

of water not shown), and which passes over a series of vessels A, at right angles to the plan of the figure, and is connected with each by the tube *a b*, in which is fixed a stop-cock to open and shut the connexion between the water-supply tube *a* and the vessel A. *c d e* is a leaden exit-tube for air, enlarged at the point *c*, and downwards, until it opens into the vessel A, thus allowing the half-inch safety-tube, *q r s*, to pass through it and down to the bottom of the vessel A, as indicated by the dotted line.

The bottles B and C (which with T, E, and O, are each of about 30 ounces capacity), contain sulphuric acid of sp. gr. 1.85, to a depth of $2\frac{1}{2}$ inches. The tube D D is about 3 feet long and 1 inch diameter, filled with fragments of pumice saturated with sulphuric acid, in which, at *ff* are indentations to prevent the sulphuric acid from draining against the corks. The bottle E contains a saturated solution of ignited carbonate of soda, and its interior is connected with that of the glass shade F, by means of the bent, and caoutchouc-jointed glass tubes *g h*.

The shade F is about 9 inches in diameter and 40 inches in height, but in some experiments, shades 16 inches in diameter and 28 in height were used. *i k* (better indicated in fig. 2, plate II) is the exit tube for the air, and is connected, externally to the shade, with an eight-bulbed apparatus, M, containing sulphuric acid. *u v*, also better indicated in fig. 2, is for the supply of water, or solutions, to the soil; and *n o* is for the escape of condensed water into the bottle O, to the bottom of which passes another glass tube *t*, by means of which the condensed water which collects in it is withdrawn.

w w is a lute-vessel of hard baked and well glazed stoneware, and is, in fact, simply a shallow dish with double concentric sides, the space between which forms a groove for the reception of the shade and of the luting material, which was mercury, and for the passage of the tubes. Fig 4, plate I, is a plan of the stoneware lute-vessel, which shows the groove widened and deepened at four equidistant points for the passage of tubes underneath the shade, and also the hole *n* for the escape of the condensed water. Fig. 5 is a vertical section of the lute-vessel from A to B of fig. 4, through two of the widened and deepened portions of the groove, and through the orifice *n*; and fig. 6 is a vertical section from C to D of fig. 4.

The bottle T contains fragments of marble, from which carbonic acid is evolved by the addition of measured quantities of chlorhydric acid, supplied by means of the tube *y*.

In 1857, blocks of slate 12 inches square and $3\frac{1}{4}$ inches thick, with a circular groove, $\frac{1}{2}$ an inch wide and 2 inches deep, adapted to the diameter of the shades, but widened and deepened for the passage of the tubes, were used instead of the stoneware lutes above described; and the condensed water was withdrawn by means of a bent tube passing from the outside beneath the shade, and extending to the surface of the mercury in the groove within the shade.

On opening the stop-cock below *a*, (fig. II, plate) water flows into the vessel A, from the reservoir with which the tube *a* is in connection. As the pressure increases, the water rises in the safety-tube *q r s*, above the level in A, and air escapes by the tube *c d e*, forces its way through the sulphuric acid in the bottles B and C, through the tube D D, containing pumice saturated with sulphuric acid, then through the solution of carbonate of soda in E, enters the shade F by the tubes *g h*, and passes out through the tube *i k*, and the bulb apparatus M, containing sulphuric acid. Calculation showed that the minimum pressure required to force the air through the apparatus was equal to that of a column of mercury 1.037 inch in height. The difference between the height of the water in A and in the safety tube *q r s*, must be equal to this amount multiplied by the specific gravity of mercury; and the height from the top of the vessel A to *r* being about 12 inches, the whole of the air passed out of A before the water ascended to *r*, and flowed out of *g*; whilst the great height of *d* prevented the water from passing over into the bottle B; an accident which unfortunately happened on a few occasions in 1857, when the air and safety-tubes were somewhat differently arranged.

When the vessel A was full of water, it was let off by a cork hole at the bottom, air being at the same time admitted by the tube *x* at the top.

The minimum pressure upon the inside of the glass shade F would be $\frac{1.0 \times 1.85}{13.6} = 0.136$ inch, 1.0 being the difference between the levels of the sulphuric acid in the bulb-apparatus M; but owing to friction, &c., the maximum pressure might be raised to double this calculated minimum.

All the Woulfe's bottles were made as air-tight as possible, by means of very good corks, which, in the cases of E and O, were covered with a cement composed of 8 parts gutta percha, 12

parts common rosin, and 1 part Venice turpentine, well melted together. The glass tube *no* was also fixed into the lute-vessel at *n* with this cement. At first, tubes of unvulcanised, but afterwards of vulcanised caoutchouc were used for the various joints indicated. The ends of the tubes *t* and *u* were fitted with caoutchouc tubing, into which pieces of solid glass rod were fitted as stoppers.

In 1857, twelve such sets of apparatus, and in 1858 a larger number, were employed. The whole were arranged side by side, on stands of brickwork, in the open air, and were protected from rain, or the too powerful rays of the sun, by a canvas awning which could be drawn over them, or withdrawn, at pleasure. In 1858, two glass cages, such as were used by M. G. Ville, and which he kindly sent over to us, were also employed.

The volume of washed air passed through the apparatus daily, was, in the earlier experiments, that of the vessel A, equal to about two and a-half times that of the shade F, but in the later ones generally twice as much, or more.

The apparatus above described has the following advantages:—

1. When it is once enclosed, and the mercury poured into the groove, the plant is entirely excluded from all external sources of combined nitrogen; and, in case of its being necessary to open the vessel, this can be done with great facility.

2. By means of the tube *no*, and the bottle O, the water which condenses within the shade is quickly removed from the atmosphere of the plant. The pan in which the pot stands, with its inward-turned rim, allows of a store of water being kept beneath the plant, which, at the same time, is protected from free evaporation. Water is very easily supplied by the tube *uv*, and the bottle O holds as much as can be condensed during several days; its contents are easily removed by means of the tube *t*, and returned, if required, by the tube *uv*.

3. A simple glass shade is more easily cleaned before commencing the experiment, and is less likely to retain combined nitrogen at its termination, than a complicated metallic framework with panes of glass cemented into it; whilst the presence of oxidisable metallic surfaces is avoided.

4. The only organic matter within the shade is the thin coating of cement by which the tube *no* is fixed into the hole *n* at the bottom of the lute; and analysis showed, that even if the whole of the cement in contact with the condensed water became decomposed, it

could only yield a fraction of a milligramme of nitrogen, whilst, experiment proved that it did not suffer sensible decomposition when exposed in the open air during a whole year.

5. During the passage of the air, the excess of pressure is upon the inside of the enclosing vessel, instead of, as in the experiments of others, upon the outside, and hence, any leakage would be from within outwards, instead of from without inwards, by which extraneous combined nitrogen might be introduced; and when the air is not passing, any pressure in the opposite direction, due to changes of temperature and barometric condition, can never exceed that required to drive air inwards through the sulphuric acid in the bulb-apparatus M.

6. The part of the apparatus which is most liable to leak, and would be the most damaged by pressure, is subjected to the minimum amount of it, the only pressure exerted upon the glass shade being the amount requisite to force the air through the bulb-apparatus M.

Lastly in reference to the arrangements for experiment, it may be observed, that the proportions of nitrogen and oxygen in the air can be only very immaterially affected by absorption under the influence of the slightly increased pressure in the vessel A; that provided the whole of the carbonic acid of the air were absorbed by the water, this would be of no consequence, arrangements being made for its artificial supply; and, that although the air may take up a considerable amount of water in the vessel A, it must lose most or all of it in passing through the sulphuric acid in the bottles B and C, and over the pumice saturated with sulphuric acid in D D, and the re-dried air will pass too rapidly through the carbonate of soda solution in E for re-saturation, whilst, as the air will be cooler before it enters the shade than within it, it will not be then so near its point of saturation.

The conditions of atmosphere provided were proved to be adapted for healthy growth, by growing wheat, barley, and beans, under the adopted conditions, but in a good garden soil, when luxuriant vegetation was the result. (See fig. 13, plate III.)

The conditions of the artificial soils were shown to be suitable for the purposes of the experiments by the fact, that plants grown in such soils, and in the artificial conditions of atmosphere, developed luxuriantly if only manured with substances supplying combined nitrogen. (See figs. 7-12, plate III.)

Taking up the Plants, Preparation for Analysis, Methods of Analysis, &c.

At the termination of growth, the glass shade was washed outside, quicksilver was poured into the groove, to displace from it the condensed water not removable by the arrangement of 1857, or collected in the bottle O of 1858, as the case might be, and the shade was then removed. The previously covered portions of the slate or stone-ware lute were then washed with pure distilled water, and the washings added to the condensed or drain-water. In the experiments of 1858 this fluid was analysed separately, but in those of 1857, it was mixed and dried down with the soil.

The pot with its contents, was removed to a clean table covered with white paper, the plants measured in all their parts, and then cut off at the surface of the soil; the roots were removed, slightly washed from soil and their character noted. The plants were then put into a small wide-mouthed bottle, generally stem and root together, but sometimes they were put into separate bottles. In the experiments of 1857 the contents of the bottles were dried in a water-bath, with a current of air, previously washed with sulphuric acid, passing through the bottle and thence through a solution of a known quantity of pure oxalic acid; but as no appreciable amount of ammonia was thus accumulated, in 1858 a little oxalic acid (in solution) was added to the vegetable matter, and the whole dried in the water-bath without the above precaution.

When dry, the vegetable matter was cut small by means of a pair of clean long scissors reaching to the bottom of the bottle, and it was afterwards still further reduced by grinding up in the mortar when mixed with soda-lime for analysis. When duplicate analyses were made, the matter was carefully divided, so as to ensure equal proportions of stem, fine leafy matter, &c., in each half; so that, if both analyses were successful, the results were mutually confirmatory, or if one portion were lost, the other represented a proportional amount of the whole.

The soil was removed from the pot to a porcelain dish, and a sufficient amount of a solution of oxalic acid added to keep it acid. The mixture was then heated on a sand-bath, stirring constantly, until most of the water was expelled, more fully dried in the water-bath, and then preserved in well-corked bottles for

analysis. The pots were pounded up; those of 1857 being preserved and analysed separately, and those of 1858 mixed with the soil before it was dried with oxalic acid. The pieces of flint at the bottom of the pot were also pounded and mixed with the soil.

For analysis, 150 to 200 grammes of the soil, pot, or mixture, were mixed with about half the volume of soda-lime, the whole put into a large combustion-tube, some soda-lime put in advance of the mixture, and then asbestos, as usual. The combustions were made in charcoal furnaces, and the ammonia collected in titrated sulphuric acid (see p. 141). When very small quantities of nitrogen were evolved, the ammonia from two or three tubes of substance was sometimes collected in the same quantity of acid, so as to diminish the error of titration. It was found, however, to be better to use very small quantities of acid, and to estimate the product of each combustion separately; for, by the former method, if any accident occurred in the second or third combustion, it involved the loss of the determination of the products previously collected.

Preparation of the Titrated Solutions.—A weighed quantity of pure, dry, carbonate of soda was dissolved in water, and water added to a given volume. As a preliminary step, the strength of some dilute sulphuric acid was tested against a given volume of the carbonate of soda solution; and from the data obtained, by further dilution a large quantity of acid was made of about the strength desired. The exact value of this acid was then ascertained. To accomplish this, a given volume of the carbonate of soda solution was put into a beaker, a little litmus added, and the mixture heated over a spirit lamp. The acid was then allowed to flow from a burette until a wine-red colour (indicating the presence of bicarbonate with carbonic acid in solution) was produced. On boiling, the blue colour is restored; acid is added until red; the boiling is repeated until the blue returns; acid again added, and so on, until the solution remains red on the addition of the last drop. The point at which the permanent change takes place in the first trial being known, the experiment is easily repeated so as to ensure great accuracy.

Thus, 50 septems of carbonate of soda solution, of which 1,000 septems contained 6.652 grammes of the salt, required, for neutralization, in six different trials—58.3, 58.2, 58.3, 58.3, 58.2, 58.2; mean 58.25 septems. Hence—

$$\frac{6.652}{1000} \times \frac{50}{58.25} \times \frac{N}{\text{NaO.CO}_2} = \frac{6.652}{1000} \times \frac{50}{58.25} \times \frac{14}{52.98} = 0.001508 \text{ gramme N.}$$

The mean of six experiments with a solution of carbonate of soda of another strength gave, in the same way, 0.0015008 gramme nitrogen. The mean, or 0.001504 gramme, was therefore adopted as the amount of nitrogen corresponding to 1 septem of the titrated acid.

As the alkali, to test against this standard acid, a solution of sugar-lime was first employed, but being found liable to constant change, due doubtless to fermentation, a solution of caustic soda was had recourse to. The burette was of small enough diameter to allow of one-tenth of a septem being read off on it, and the alkali-solution was so dilute that it required about 3 septems of it to neutralise 1 septem of the acid. Hence, 1 septem of the alkali-solution corresponded to only about half a milligramme of nitrogen, and the probable error of reading would therefore be only about one-twentieth of a milligramme—an amount much less than would be admissible as error of analysis. In the case of the sugar-lime solution it was found necessary to test its strength against that of the acid every day that it was employed. But the soda-solution, if properly prepared, and well preserved, remained for months unchanged.

Amount and measurement of the Titrated Acid used in Nitrogen Determinations.—It was desirable that at least 3 times as much acid should be used as would be neutralised by the ammonia formed. The acid being more concentrated than the alkali, it required a more exact method of measurement; and pipettes, of which the diameter at the point of reading off was comparatively small, were therefore employed; in the construction of which, care was taken to maintain the same relation of the diameter of the neck at the point of reading to the entire volume in instruments of different sizes—a condition seldom observed by makers of pipettes. When the quantity of nitrogen involved in an analysis was very small (as is the case of the soils and pots in the experiments without nitrogenous manure), only about 6 septems of the titrated acid, measured in a small pipette with a very narrow neck were used, the exact volume of which was of no consequence, it being only essential to ascertain its exact value expressed in septems of the titrated alkali-solution. When the amount of nitrogen involved was larger, as, for example, when

grains were to be analysed, sufficient accuracy was attained when the substance experimented upon contained 5 to 8 milligrammes, or more, of nitrogen.

Combustion-tubes, Bulbs, &c.—The tubes used in the determinations of nitrogen in the soils, pots, &c., were about 3 feet long and about 1 inch in diameter. The bulb-apparatus, was capable of holding two and a-half to three times as much fluid as that usually employed; but the central and lowest bulb, and particularly its tubular connexions with the other bulbs, were very small, so that a small quantity of liquid could close the passage. This arrangement was necessary, owing to the small quantity of acid frequently used, and the large amount of water driven off in the combustion from the large quantities of soil and soda-lime. For the combustion of the experimentally grown plants, smaller tubes were employed; and for the seeds, &c., ordinary combustion-tubing was used.

Soda-lime.—Before use, the soda-lime was ignited with 2 per cent. of pure sugar, in order to ensure its freedom from ammonia yielding matter. It was then slaked with pure distilled water, dried, and kept in well-corked bottles.

Accuracy of the Method for the Determination of Nitrogen by Combustion with Soda-lime, &c.—In order to ascertain the accuracy of the method, some experiments were made upon the determination of small and known quantities of nitrogen, mixed with large quantities of soil previously freed from combined nitrogen as in the preparation of the soils for the plant-experiments. The nitrogenous substance taken was the powdered crystals of purified quadroxalate of ammonia—

The results were—

Experiment 1.—50 grammes of prepared soil, mixed with quadroxalate, containing by calculation 0·0024 gramme nitrogen, gave on burning with soda-lime, and determining as above 0·0027 gramme.

Experiment 2.—100 grammes of soil, mixed with quadroxalate, equal by calculation to 0·0035 gramme nitrogen, gave on combustion 0·0037 gramme.

The error of analysis was, therefore, three-tenths of a milligramme of nitrogen with the 50 grammes, and two-tenths with

the 100 grammes of soil. These results were obtained at the commencement of the inquiry, with comparatively large quantities of the titrated acid, and before experience had suggested the precautions to be adopted to reduce the errors of determination to the minimum. They may hence be taken as examples of the maximum errors of analysis, but they are less than would affect the bearing of the results in the investigation on the question of assimilation.

Qualitative Examination for Nitric Acid.—The indigo test, as recently refined by Boussingault,* and the protosulphate-of-iron test, were both employed. When nitric acid was sought for and not found, the negative result was, if practicable, always controlled by the addition to some of the substance under examination of a quantity of nitric acid (in the form of nitrate) less than could affect any conclusions to be drawn from the fact of its presence or absence in the substance in question; and, in all such cases, the re-examination showed the presence of nitric acid.

The method of Boussingault was much more delicate than the protosulphate-of-iron test; but, on the other hand, the latter was much less liable to give deceptive indications, dependent on other circumstances than the presence of nitric acid. In using the protosulphate test, the aqueous extract of the substance under examination (after destroying any organic matter by boiling with permanganate of potassa, &c.), was evaporated to a small volume with excess of fixed alkali, then transferred to a test-tube, and further evaporated till only a few drops remained.. A considerable excess of concentrated sulphuric acid was then added, and on the surface of the liquid, a concentrated solution of protosulphate-of-iron was carefully poured, without agitation, by means of a small pipette with a mouth of almost capillary fineness. The characteristic tinge indicated the presence of nitric acid.

Conditions of Experiment requiring Collateral Investigation.

Under this head it is proposed to consider:—1. The possible influence of ozone in promoting the formation of nitrogenous compounds from free nitrogen, either within or in connexion with the

* Ann. Ch. Phys., vol. xlviii (1856), p. 153 et seq.

plant, or within the soil, either directly, or in connexion with the organic matter of the plant. 2. Whether or not there be an evolution of free nitrogen in the decomposition of nitrogenous organic matter. 3. Whether, under the circumstances of the experiments, there would be likely to be a formation of nitrogenous compounds, by the mutual action of nascent hydrogen (evolved by decomposing organic matter) and free nitrogen.

With regard to the first point, ozone may be supposed to occur within the cells and intercellular passages of the plant, either in the gaseous state or in solution, or it may be simply around the plant without existing in its structures. It may be a product of the action of the sun's rays, by virtue of which carbonic acid is decomposed and oxygen evolved, or it may result from other actions to which reference will presently be made.

To ascertain experimentally, if possible, whether ozone were present within the cells or intercellular passages, the gases obtained by exhausting plants placed in water freed from air by boiling, were examined, but no ozone was detected. Other experiments were also made, in which about an ounce respectively of green wheat, barley, oats, beans, and clover, were placed in 500 cub. cent. of carbonic acid water, and the whole subjected to sunlight, but though from 100-200 cub. cent. of gas were obtained, which contained sufficient oxygen to inflame a glowing taper, test-paper placed in it did not indicate a trace of ozone. Granting, however, that ozone may, under certain circumstances, be a product of the decomposition of carbonic acid within the plant, it is still a question whether such conditions would be favourable to its oxidating free nitrogen; an action which will obviously be dependent on the intensity of the reducing power of other substances present. The investigations of Schönbein and others appear to show that, under certain circumstances, nitric acid may be formed by the mutual action of ozone and free nitrogen; but the question is, whether these circumstances are presented in the cells and intercellular passages of growing plants, a point which we have sought to study by the examination of the gases they contain under various circumstances.

Plants, or parts of plants, were put into a flask filled with water which had been freed from air by boiling; a cork, through which a bent glass tube was passed, was then pressed into the flask, filling the tube with the displaced water. The flask was then

placed over a lamp, the water boiled, and the expelled water and gas collected over mercury, the boiling being continued until the vapour produced expelled most of the water collected over the mercury. The gas so obtained, in numerous experiments, consisted entirely of nitrogen and carbonic acid, without any oxygen; from which it is obvious that the reducing power of the carbon compounds in the vegetable cells, was sufficient, under the circumstances, to consume all the oxygen (or ozone) that might be present. But the high temperature at which the experiment was conducted, must have tended very much to increase this action.

In all subsequent experiments a different plan of operation was adopted. The plants were put into a tall glass vessel, 14 inches high, and 1.75 inch in diameter (fig. 7, plate I), the mouth of which was fitted with a long cork, previously well-boiled in bees'-wax, through which two glass tubes, *a* and *b*, are inserted. The vessel being filled with water previously well-boiled and cooled without access of air, the plant is put in and well-shaken to remove adherent air-bubbles. The cork is then forced in, taking care that both the tubes become filled with water, and that no air remain in the vessel. As a further security for tightness, a piece of thick caoutchouc tubing may be drawn over the neck of the vessel, projecting upwards a little above the cork, and the cup thus formed, partly filled with melted wax, forming a layer over the cork and its joints. A funnel is then attached to the tube *b*, by means of a caoutchouc tube which can be closed by a strong pinch-cock. Water being admitted through the funnel into the tube *b*, the tube *a* becomes filled, and it is then brought into connexion, by means of a glass tube and caoutchouc joint fitted with a pinch-cock, with a vessel filled with quicksilver. The connexion being opened, the quicksilver is allowed to flow from the vessel by means of a long tube, of more than barometric length, fitted into the lower part of it, thus forming a Torricellian vacuum in the mercury vessel. The gas from the plant passes over into the vacuum, and by a simple arrangement is collected in an eudiometer tube for examination.

The following table (VIII) shows the amount and composition of the gas obtained in the manner above described, from different plants, *in the shade* :—

TABLE VIII.

Date.	Description.	Total gas col- lected ; cub. cents.	Per cent.			
			Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and carbonic acid.
Wheat; 1858.						
June 16.	Whole plant.....	57·0	77·72	2·28	20·00	22·28
June 17.	Whole plant.....	55·3	77·94	5·06	17·00	22·06
June 16.	Whole plant.....	57·0	78·60	1·75	19·65	21·40
June 16.	Whole plant.....	55·7	77·38	3·23	19·39	22·62
June 16.	Whole plant.....	65·7	82·50	0·30	17·20	17·50
Barley; 1857.						
June 24.	Whole plant.....	8·6	85·12	3·93	10·95	14·88
June 24.	Whole plant.....	20·9	81·48	1·97	16·55	18·52
Beans; 1858.						
June 17.	{ Whole plants coming into flower }	54·3	79·74	5·16	15·10	20·26
June 17.	{ Whole plants coming into flower }	41·5	86·74	4·10	9·16	13·26
June 17.	{ Whole plants coming into flower }	52·5	80·38	4·38	15·24	19·62
June 17.	{ Whole plants coming into flower }	50·4	84·33	4·36	11·31	15·67
Clover; 1857.						
Aug. 10.	Heads	47·7	85·61	6·00	8·39	14·39
Aug. 10.	Stems and leaves	59·8	83·23	2·33	14·44	16·77
Aug. 11.	Heads	91·0	87·15	1·89	10·96	12·85
Aug. 11.	Stems and leaves	42·3	78·32	1·31	20·37	21·68

These results show that the reducing power of certain of the carbon compounds of the plant was sufficient to convert nearly the whole of the oxygen (or ozone) present, into carbonic acid, when in the shade.

Over 100 exhaustions were made, precisely as in the case of the last experiments, with the exception that the plants were exposed, during the whole process, to the direct rays of the sun. Table IX exhibits a few of the results obtained :—

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TABLE IX.

Date.	How manured, &c.	Total gas collected; cub. cents.	Per cent.			
			Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and carbonic acid.
Wheat (whole plants); 1858.						
June 22.	Unmanured	44·4	73·65	21·17	5·18	26·35
June 23.	Unmanured	34·8	77·01	21·26	1·73	22·99
June 30.	Unmanured	44·1	72·79	20·86	6·35	27·21
June 22.	{ Mineral and ammoniacal manure }	54·5	73·76	21·29	4·95	26·24
June 23.	{ Mineral and ammoniacal manure }	42·1	78·15	15·44	6·41	21·85
June 25.	{ Mineral and ammoniacal manure }	37·2	78·76	19·09	2·15	21·24
Grass (whole plants; second crop); 1857.						
Aug. 15.	{ Mineral and ammoniacal manure }	39·0	82·10	16·19	1·71	17·90
Aug. 15.	{ Mineral and ammoniacal manure }	47·8	77·08	15·35	7·57	22·92
Aug. 15.	{ Mineral and ammoniacal manure }	41·6	76·56	21·46	1·98	23·44
Aug. 17.	{ Mineral and ammoniacal manure }	39·9	75·07	23·39	1·54	24·93
Aug. 18.	{ Mineral and ammoniacal manure }	36·8	79·88	15·19	4·93	20·12
Aug. 18.	{ Mineral and ammoniacal manure }	42·3	80·23	15·97	3·80	19·77
Beans; 1858.						
July 12.	{ Mineral manure; almost podding }	44·3	71·11	18·28	10·61	28·89
July 12.	{ Farm-yard manure; almost podding }	45·8	73·14	10·26	16·60	26·86
July 15.	{ Unmanured; almost podding }	25·9	82·63	15·83	1·54	17·37
July 15.	{ Mineral and ammoniacal manure; almost podding.... }	30·9	70·55	20·71	8·74	29·45

The general accordance in the proportions of nitrogen throughout this series, together with their general approximation to the amounts observed in the preceding series, and the consequent similarity in the range of the sums of the two remaining gases—

carbonic acid and oxygen—point to the character of the change by virtue of which the proportion of carbonic acid is diminished, and that of oxygen increased. The variations are, nevertheless, somewhat considerable; nor do we feel confidence in referring them to any other than accidental causes. There can be no doubt, however, that the carbonic acid shown to exist in the plants in the shade yields the oxygen evolved in the sunlight.

The results which follow will show the influence of the time of action of the sunlight on the plant, upon the relative proportions of carbonic acid and oxygen.

Duplicate quantities of plant were operated upon, both being prepared in the shade; the vessels were then excluded from the light by means of a thick paper covering; and, in this condition, each was attached to a Torricellian exhaustor (the apparatus above described). The covering being then removed from one of the vessels, exposing it and its contents to the direct rays of the sun, and the other remaining covered, the exhaustion of both was then commenced immediately, and the action continued for half-an-hour. The following results were obtained in this manner:—

TABLE X.

Date.	Description of plant.	Conditions during exhaustion.	Total gas collected.	Per Cent.			
				Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and carbonic acid.
(1858)			cub. cents.				
	Beans.	{ In dark ..	25·7	66·93	2·33	30·74	33·07
		{ In sunlight	36·4	69·78	8·24	21·98	30·22
July 22.	Oats.	{ In dark ..	28·3	81·63	3·53	14·84	18·37
		{ In sunlight	25·9	70·27	13·13	16·60	29·73
July 23.	Oats.	{ In dark ..	26·4	73·11	8·33	18·56	26·89
		{ In sunlight	22·7	72·25	16·74	11·01	27·75
July 28.	Oats.	{ In dark ..	27·4	68·25	5·11	26·64	31·75
		{ In sunlight	29·2	67·47	19·86	12·67	32·53
July 23.	Oats.	{ In dark ..	31·4	77·39	6·69	15·92	22·61
		{ In sunlight	21·7	76·50	16·59	6·91	23·50

The results clearly indicate the ready transformation, in sunlight, of carbonic acid into a solid carbon-compound and free oxygen; and they have an important bearing on the question under con-

sideration, showing, as they do, the powerful reducing action exerted upon the carbonic acid under the influence of the sun's rays.

The experiments next cited were arranged to show whether the reduction of the carbonic acid was more probably due to the action of the sunlight at the moment of the passage of the gas through the walls of the cell, or whether the oxygen were liberated within the growing cell. Duplicate portions of plant were prepared exactly as in the experiments last referred to, and both were kept in the dark for some time before commencing the exhaustion; then, one being still covered, and the other exposed to sunlight, both were submitted to exhaustion for four or five minutes only, increased quantity of plant being operated upon in order to obtain sufficient gas during this short period. Table XI gives the results so obtained :—

TABLE XI.

Date.	Description of plants.	Conditions during exhaustion.	Total gas collected.	Per Cent.			
				Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and carbonic acid.
(1858)			cub. cents.				
July 30.	Oats.	{ In dark ..	41·7	72·42	3·6	23·98	27·58
		{ In sunlight	42·5	72·23	4·71	23·06	27·77
July 30.	Oats.	{ In dark ..	55·7	71·46	3·23	25·31	28·54
		{ In sunlight	43·3	69·98	3·23	26·79	30·02
July 30.	Oats.	{ In dark ..	37·9	83·11	6·86	10·03	16·89
		{ In sunlight	38·5	77·14	9·09	13·77	22·86
July 31.	Oats.	{ In dark ..	34·4	78·49	7·27	14·24	21·51
		{ In sunlight	41·8	75·84	7·89	16·27	24·16

It would appear that carbonic acid can pass through the cell-wall under the influence of sunlight without suffering decomposition, and hence that the oxygen yielded by a plant which has been for some time exposed to the direct rays of the sun, existed as such in the cell before the exhaustion. The slight preponderance of oxygen in the gas exhausted in sunlight was doubtless due to its action upon the carbonic acid within the cell during the short period of the operation, just as when the plant is subjected to ordinary atmospheric pressure.

In order to bring out more clearly the influence of sunlight before the exhaustion, experiments were made in which duplicate quantities of plant were each covered for some time, the paper was then removed from one, and then, after an interval of from 20 to 30 minutes, the exhaustion of both was commenced, and continued for 10, 15, or 20 minutes. The following results were obtained:—

TABLE XII.

Date.	Description of plant.	Conditions before and during exhaustion.	Total gas collected.	Per cent.			
				Nitrogen.	Oxygen.	Carbonic acid.	Oxygen and carbonic acid.
			cub. cents.				
July 31 ..	Oats.	{ In dark ..	24·0	77·08	3·75	19·17	22·92
		{ In sunlight	34·5	68·69	24·93	6·38	31·31
August 2.	Oats.	{ In dark ..	18·6	68·28	10·21	21·51	31·72
		{ In sunlight	39·2	67·86	25·25	6·89	32·14
August 2.	Oats.	{ In dark ..	30·7	76·87	8·14	14·99	23·13
		{ In sunlight	26·5	69·43	27·17	3·40	30·57
August 2.	Oats.	{ In dark ..	17·0	79·41	7·65	12·94	20·59
		{ In sunlight	28·6	76·22	18·53	5·25	23·78
August 3.	Oats.	{ In dark ..	29·8	81·88	6·38	11·74	18·12
		{ In sunlight	32·1	66·36	30·53	3·11	33·64
August 3.	Oats.	{ In dark ..	11·6	65·52	6·90	27·58	34·48
		{ In sunlight	23·1	70·56	20·35	9·09	29·44
August 3.	Oats.	{ In dark ..	17·0	80·00	5·88	14·12	20·00
		{ In sunlight	19·7	73·10	22·33	4·57	26·90

The comparison of these results with those in Table XI, together with a consideration of the fact that, as soon as the pressure was removed, the gas was evolved from all parts of the leaf, and not from the surrounding water, shows that the oxygen must have been liberated and retained within the cells until the instant of exhaustion.

The results of this part of the enquiry may be briefly summed up as follow:—

1. Carbonic acid, within growing vegetable cells and intercellular passages, suffers decomposition very rapidly on the penetration of the sun's rays, oxygen being liberated, and remaining within the plant, or being evolved from it.

2. Living vegetable cells, in the dark, or not penetrated by the direct rays of the sun, consume oxygen very rapidly, carbonic acid being formed.

3. Hence, the proportion of oxygen must vary greatly, according to the position of the cell, and to the external conditions of light; and it will oscillate under the influence of the reducing force of carbon-matter (forming carbonic acid) on the one hand, and that of the sun's rays (liberating oxygen and forming carbon-compounds containing less oxygen than carbonic acid) on the other. Both actions may go on simultaneously, according to the depth of the cell; and, according to the laws in conformity with which the diffusion of gases, and their passage through tissues, take place, the oxygen of the outer cells would be continually penetrating to the deeper cells, and there oxidising carbon-matter, whilst carbonic acid would pass in the opposite direction, and be decomposed under the influence of sunlight in the outer cells; and the once outer cells, as they become more deeply buried, may gradually pass from the state in which the sunlight is the more powerful, to that in which the carbon-matter is the more powerful reducing agent—from the state in which there is a flow of carbonic acid to them, and of oxygen from them, to that in which the reverse action takes place. Thus, oxidised products—acids, saccharine matter, &c.—may be formed in the deeper cells, and in the outer cells more highly carbonised substances, which, in their turn, become oxidised when buried deeper.

4. The great reducing power operating in those parts of the plant where ozone is most likely, if at all, to be evolved, seems unfavourable to the oxidation of nitrogen; that is, under circumstances in which carbon-matter is not oxidised, but, on the contrary, carbonic acid is reduced, and, when beyond the influence of the direct rays of the sun, the cells seem to supply an abundance of the more easily oxidised carbon-matter, should free oxygen, or ozone, be present. Further, on the assumption that nitrates are available as a source of nitrogen to plants,* if it were admitted that nitrogen was oxidated within the plant, it must be supposed (as in the case of carbon) that there are conditions under which an oxygen-compound of nitrogen may be reduced

* In reference to this point, it may be mentioned that several specimens of green wheat and grass, which had been liberally manured with nitrate of soda, were examined for nitric acid, but no trace of it was found in them.

within the organism, and that there are others in which the reverse action, namely, the oxidation of nitrogen, can take place.

5. Bearing upon the questions under consideration, but illustrated by experiments to be referred to further on, it may be stated, that so great is the reducing power of certain carbon-compounds of vegetable matter, that when the growing process has ceased, and all the free oxygen in the cells has been consumed, water is decomposed, and hydrogen evolved; but, as the action does not continue long, it would appear either that the cell-matter loses its reducing power after being slightly oxidized, or, more probably, that the cell provides a certain amount of matter more easily oxidized than the remainder.

The suggestion arises, whether ozone may not be formed under the influence of the powerful reducing action of the carbon-compounds of the cell on the oxygen eliminated from carbonic acid in sunlight, rather than under the direct action of the sunlight itself—that is, in a manner analogous to that in which it is ordinarily obtained by allowing oxygen to come into incomplete or only instantaneous contact with phosphorus? Certain carbon-compounds of the vegetable cell have a great affinity for oxygen in the dark, and the oscillations of the affinities (due to the degree of light and to the depth of the cell) would afford conditions somewhat similar to those under which ozone is produced in the presence of phosphorus. But, even were the action as here supposed, it may be questioned whether the ozone would not be at once destroyed in contact with the carbon-matter present. It is more probable, however, that the ozone said to be observed in the vicinity of vegetation, is due to the intense action of the oxygen of the air upon minute quantities of volatile hydro-carbons emitted by the plants, than to any action within the cells; and it is in favour of this view, that ozone has been observed most readily in the vicinity of such plants as emit freely essential oils.

If ozone be found in any way in connexion with the growing plant, it might be supposed to act in an indirect, if not in a direct manner, as a source of combined nitrogen in experiments on the question of the assimilation of free nitrogen by plants—(1) by oxidating the nitrogen dissolved in the water within the apparatus, (2) by forming nitrates in contact with the moist, porous, and alkaline soil, (3) by oxidating the free nitrogen in the cells of the older roots or that evolved in their decomposition.

With a view to the points above suggested, experiments were made to ascertain the influence of ozonous air upon organic matter, and on certain porous and alkaline bodies, under various circumstances. For the generation of the ozonous atmosphere, three glass carboys, each of about 40 litres capacity, were fitted with stone-ware stoppers, through which glass tubes passed, connecting the three together, the joints being made with calcined gypsum cement. The bottom of each vessel was covered with water to the depth of from a quarter to half an inch, so that, on pieces of phosphorus being dropped in, they were partly covered by the fluid. Another tube, which could be opened or closed at pleasure, was fixed through each stopper for the supply of water and fresh phosphorus, as needed. A gasometer, of 2 cub. feet capacity, was connected by a glass tube with the first of the three vessels, by which means air could be forced through them in a continuous stream. The air, on passing out, was led first through a wash-bottle, and then into a glass vessel, from which, by means of eleven glass tubes passing from it, it was distributed into as many bottles, containing, respectively, the substances to be submitted to the action of the ozone, each bottle being fitted with an exit tube, in which were fragments of pumice, saturated with sulphuric acid. The following were the substances or mixtures operated upon:—

(1) $\frac{3}{4}$ lb. of ignited soil, moistened with 100 cub. centims. water; this being just sufficient, to make it slightly coherent.

(2) $\frac{3}{4}$ lb. of ignited soil, 300 cub. centims. water, 2·5 ounces boiled starch, and 2·5 ounces dry starch.

(3) $\frac{3}{4}$ lb. of ignited soil, 200 cub. centims. water, and 2·5 ounces sawdust.

(4) 2·5 ounces sawdust, and 100 cub. centims. water.

(5) $\frac{3}{4}$ lb. of ignited soil, 200 cub. centims. water, and 2·5 ounces bean-meal.

(6) $\frac{3}{4}$ lb. of ignited soil, 150 cub. centims. water, and 2·5 ounces bean-meal.

(7) 2·5 ounces bean-meal, and 50 cub. centims. water.

(8) 1lb. garden soil.

(9) $\frac{3}{4}$ lb. of slaked lime, and 2·5 ounces bean-meal, made slightly pasty with water.

(10) $\frac{3}{4}$ lb. of slaked lime, some starch, and sawdust, made slightly pasty with water.

(11) 2·5 ounces of boiled starch, 2·5 ounces fresh starch, and 200 cub. centims. water.

All the bottles stood before a window where the sun shone directly upon them most of the day, as it did also for some hours on the balloons. The experiment commenced in April, and continued through the summer and part of the autumn, and the thermometer in the room frequently stood at 25° to 29° C. About 9 o'clock every morning, and once or twice more during the day, the cylinder of the gasometer was raised, and by its gradual depression a slow current of air was passed through the apparatus during about two hours. The amount of ozone passed was so great, that vulcanised caoutchouc tubing, first used for the joint with the tube of the wash-bottle, was cut off by the passage of a few gasometerfuls of air; and the joint was then made by means of a piece of larger glass tubing passed over the point of contact of the tubes to be connected, and closed at the ends with rings of cork well fitted upon the smaller tubes. Every three or four days a small piece of phosphorus was dropped into each balloon.

Until the beginning of July the wash-bottle was filled with large lumps of pumice, and about half full of a solution of caustic potass, which, by the rapid bubbling of the ozonous air through it, kept the pumice moist. An examination of this liquid, with the washings of the pumice, failed to show any trace of nitric acid. From the beginning of July, the alkaline wash was replaced by pure water, and, at the termination of the experiment, these washings also gave no indication of nitric acid.

At the termination of the experiment, a portion of each substance or mixture was exhausted with water, and the extract concentrated by boiling, after the addition of permanganate of potassa to destroy the organic matter. The excess of permanganic acid was removed by carbonate of lead, and the clear solution filtered off and tested for nitric acid; but in no case, excepting that of the garden soil (which examination showed to contain it before being subjected to the action) was there any indication of its presence.

It is not, indeed, hence inferred, that under no circumstances can nitric acid be formed by the action of ozone, on nitrogenous compounds of the ammonia class, or the nascent nitrogen evolved from them, or even in connexion with non-nitrogenous bodies, or porous substances permeated with gaseous nitrogen, or in the atmosphere itself; or that the nitric acid in soils is not in part due to some of these actions. But, considering the negative result with large quantities of ozonous air, acting upon

organic matter, soil, &c., in a wide range of circumstances, and for so long a period, it is believed that no error in the results of the experiments on the assimilation of free nitrogen by plants will arise from the action of ozone upon free nitrogen, providing to the plants an unaccounted supply of combined nitrogen.

It may be here observed that, if from the recent results of Schönbein, on the formation of nitrogen-compounds under the influence of evaporation, we are to conclude that the evaporation of water by plants during growth is a constant source of such formation, it would be difficult to arrange an experiment on the question of the assimilation of free nitrogen by plants that would not be open to objection; for there must then be always a gain of combined nitrogen in the experiment due to the action supposed. Or, if such formation be the constant result of evaporation from the surface of the globe (whether land or water), the products can hardly be supposed to become in any material degree distributed through the atmosphere: for, independently of the character and condition of the products, the amount of combined nitrogen brought down from it by aqueous deposition in various forms is too small in proportion to that annually available for the vegetation of a given area, to give countenance to such a supposition.

Numerous experiments were made to determine whether free nitrogen was evolved during the decomposition of nitrogenous organic compounds. Two obvious methods of investigation presented themselves:—1, to allow the decomposition to take place under circumstances in which any free nitrogen evolved might be collected and estimated; 2, to collect and estimate the compounds of nitrogen formed, and reckon the loss of nitrogen as free nitrogen evolved.

Reiset, operating according to the first of these methods, submitted nitrogenous animal and vegetable substances to decomposition under an inclosing vessel, into which he passed oxygen as that of the air was consumed, and the result was an increase in the amount of nitrogen. Operating according to the second method, M. G. Ville concluded that, in several cases, about one-third of the nitrogen of the substance submitted to decomposition was evolved in the free state. Again, as already shown, M. Boussingault found a loss of nitrogen in his experiments on the assimilation of free nitrogen by plants when he used nitrogenous organic matter as manure.

In our own experiments, a given weight of nitrogenous organic matter, the percentage of nitrogen in which was determined, was mixed with burnt, washed, and re-ignited soil, or pumice, and put into a bottle of about 360 cub. cents. capacity (see B, plate I, fig. 8), and a proper quantity of water added. The bottle was closed with a cork, through which two bent glass tubes passed, externally in opposite directions, one connected with an eight-bulbed apparatus, A, containing sulphuric acid, and the other with a similar apparatus, C, containing a solution of oxalic acid, from which passed a bent tube, extending, through a cork, to the bottom of a second bottle, D, containing sulphuric acid; and through the cork of the bottle D another tube, E, also passed, but did not dip into the acid. On drawing air at E, a current was established inwards through the sulphuric acid in A, by which it was washed free from ammonia, through the bottle B with the decomposing organic matter, through the oxalic acid in C, by which the absorbable gaseous products were retained, and so on. At the termination of the experiment, the combined nitrogen remaining in B, and that absorbed by the oxalic acid in C, being determined, the amount of nitrogen given off in the free state was estimated by difference.

In 1857, six experiments were made, two with wheat-meal, two with barley-meal, and two with bean-meal, known quantities of each being mixed, respectively, with about 100 grammes of prepared soil, or about 60 grammes of prepared pumice, the mixtures filling the bottles B to the depth of about two inches, and sufficient water being added to bring the mass into an agglutinated condition. The six sets of apparatus were placed before a large window, where, during the middle of the day, the sun shone directly upon them. Several litres of air were drawn, by the mouth, through each apparatus daily; and the experiment was continued from June 10 to October 8. After a day or two, the gas had a more or less disagreeable taste, and the odour of decomposing organic matter. The following statement condensed from the notes taken, shows the condition of the several mixtures at the termination of the experiment:—

1. Wheat-meal and pumice.—Slightly mouldy; odour of decomposing organic matter; quite moist, particles of pumice adhering together.

2. Wheat-meal and soil.—Slightly mouldy on the surface;

odour as No. 1; the mass moist, but not sufficiently so for the particles of soil to agglutinate.

3. Barley-meal and pumice.—Not mouldy; odour like Nos. 1 and 2, but more intense, and sour, like fermenting malt; the mass wet and clammy.

4. Barley-meal and soil.—Not mouldy; odour like No. 3; sufficiently moist to agglutinate.

5. Bean-meal and pumice.—A little mould on the surface; odour very disagreeable and putrescent; the mass wet and clammy.

6. Bean-meal and soil.—Very similar to No. 5, but rather more wet and pasty.

In every case, carbonic acid was evolved on the addition of oxalic acid to the mass, preparatory to evaporating to dryness; and the most from the bean-meal with soil. A known proportion, about one-half of each dried mass, was burnt with soda-lime; and the remainder reserved for the determination of nitrates, if present. However, the aqueous extract of the mass in no case indicated nitric acid; though, on then adding 0·001 gramme of nitric acid to the residual mass, and re-extracting with water, its presence was always indicated. The following Table gives the numerical results of the six experiments:—

TABLE XIII.

Substances submitted to experiment.				Nitrogen after decomposition.			
Description of organic matter.	Description of matrix.	Quantity of meal taken.	Quantity of nitrogen.	Total by soda-lime.	Not recovered.		
					Actual quantity.	Per cent.	
		grms.	grms.	grms.	grms.		
1. Wheat-meal.	Ignited pumice	2·0585	0·0370	0·0338	0·0032		8·51
2. Wheat-meal.	Ignited soil ..	2·1282	0·0383	0·0335	0·0048		12·53
3. Barley-meal.	Ignited pumice	2·2495	0·0380	0·0368	0·0012		3·16
4. Barley-meal.	Ignited soil ..	2·0980	0·0355	0·0309	0·0046		12·96
5. Bean-meal ..	Ignited pumice	2·0650	0·0803	0·0741	0·0062		7·72
6. Bean-meal ..	Ignited soil ..	2·0800	0·0809	0·0823	(+ 0·0014)		+ (1·73)

With one exception (in which the gain of nitrogen is within the range of error of analysis), all the experiments show that a part of

the nitrogen of the decomposing organic matter passed into a state in which it could not be estimated by the soda-lime process. Neither did it exist as nitric acid. It would appear, therefore, that there was an evolution of free nitrogen. But, although so large a proportion of the total nitrogen passed off in some form, yet scarcely a trace of ammonia was given off from the mass; for, on distilling the oxalic acid in C with an excess of caustic potassa, in only one case (that of the bean-meal and pumice) was any ammonia indicated, and then it was equal to only 0.002 gramme nitrogen.

The experiments, the results of which are yet to be recorded, were arranged with some reference to the question of the character and extent of the decomposition, and of the part played by water in the process.

In 1858, a set of nine experiments was made, embracing a wide range of conditions as to moisture, in some of which were included the circumstances of germination, early growth, and subsequent decay of the products, and in which the intermediate stages and final extent of decomposition were more closely considered, the former by periodical inspection, and observation on the character of the odour, and the latter by a further examination of the remaining products. Although soil and pumice indicated no special difference of result, both were still used; about 175 to 200 grammes of the former, or 120 to 150 grammes of the latter being employed. Reference to Tables XIV and XV will sufficiently show the other conditions of the several experiments. The description of apparatus was the same as that used in 1857 (fig. 8).

The mixtures: with 50 cub. cent. of water added were in the condition of a rather moist soil; those with 40 cub. cent. were much drier, having no tendency to agglutination; and those with 100 cub. cent. were very wet, having some free water above the solid matters. The seeds sown with 50 cub. cent. germinated in a few days, and the bottles, B, were soon filled with vegetable matter (the beans especially developing a very large amount of root, as well as stemmy and leafy matter); but those with 100 cub. cent. water did not germinate, but in a few days showed decomposition. The mixtures of meal and soil also soon showed signs of decomposition, though the odour from them was less foul than from the whole seed and 100 cub. cent. water, which, throughout, were the most disagreeable; and it may be observed that the water at first resting above the solid matters disappeared so rapidly, with active decomposition, as to lead to the conclusion

that some of it was consumed in the process. There was in no case any ozone re-action to test paper. With these few general remarks, we may omit here a record of the detailed observations periodically made on the progress of the decomposition; adding only, that on July 1st (the experiments having commenced in January) 100 cub. cent. of water were added to each, and on August 28th, when the experiment was terminated, there still remained, in all the cases, a layer of from $\frac{1}{4}$ to $\frac{1}{2}$ an inch of fluid over the solid matter, the condition of which was as follows:—

Wheat (*a*)—Seeds, in soil, with 50 cub. cent. water: very little odour, and not unpleasant; supernatant fluid colourless; the organic matter thoroughly decomposed, only slight remains of stems and leaves being visible. The addition of oxalic acid to the mass preparatory to evaporation to dryness, caused a copious evolution of carbonic acid.

A similar evolution of carbonic acid took place in every one of the experiments.

Wheat (*b*)—Seeds, in pumice, with 100 cub. cent. water: supernatant fluid colourless; a disgusting mouldy odour, the form of the grain retained, but the contents disappeared, and the husks filled with fluid.

Wheat (*c*)—Meal, in soil, with 40 cub. cent. water: supernatant fluid of a yellowish colour and muddy; the mass emitted a foul, disagreeable odour, though less intense than that of the corresponding barley.

Barley (*a*)—Seeds, in soil, with 50 cub. cent. water: the organic matter thoroughly decomposed; stems, roots, and leaves no longer distinguishable; other conditions much as wheat (*a*).

Barley (*b*)—Seeds, in pumice, with 100 cub. cent. water: supernatant fluid clear; the pumice covered with a black coating of organic matter; the odour of the air above the mixture exceedingly disgusting, resembling that of decaying human excrements; traces of sulphide of hydrogen perceptible; the form of the seeds preserved, but the husks contained only fluid.

Barley (*c*)—Meal, in soil, with 40 cub. cent. water: supernatant water yellowish; odour musty, but not very disagreeable; very slight traces of organic matter perceptible.

Bean (*a*)—Seeds, in soil, with 50 cub. cent. water: the organic matter well decomposed; odour musty.

Bean (*b*)—Seeds, in pumice, with 50 cub. cent. water: only

very indefinite skeletons of stems, leaves, and roots remaining; odour musty, but not disagreeable.

Bean (c)—Meal, in soil, with 40 cub. cent. water; supernatant fluid slightly yellow; odour musty, but not offensive.

After drying with oxalic acid, any slight remains of organic matter had become brittle; and in every case, excepting where 100 cub. cent. of water had been originally added (when the husks remained visible), the mass had the appearance of clean soil, or pumice, without organic matter.

In a few instances, the sulphuric acid in D became slightly brown, indicating the passage into it, through the oxalic acid, of some more complex carbon-compound than carbonic acid; and we have observed phenomena of a similar kind in some other cases.

The following Tables, XIV and XV, show the conditions of the several experiments, and the numerical results obtained, the former relating more particularly to the amount and proportion of the original *carbon*, and the latter to those of the original *nitrogen*, remaining after the decomposition, or given off during the process :—

TABLE XIV.

Substances involved in the experiment.				Weight of organic matter.		Carbon.			
Organic matter.		Matrix.	Water added.	Fresh.	Dry.	Before decomposition.	After decomposition.	Loss in decomposition.	
Description.	Condition.							Actual quantity.	Per cent.
			cub. cent.	grms.	grms.	grms.	grms.	grms.	
1. Wheat	a. 171 seeds	Ignited soil	50	8·0475	6·7438	3·1089	0·9274	2·1815	70·17
	b. 171 seeds	Ignited pumice	100	8·0715	6·7639	3·1182	0·9178	2·2004	70·56
	c. Meal.....	Ignited soil	40	9·8810	8·2803	3·8172	1·3199	2·4973	65·42
2. Barley	a. 163 seeds	Ignited soil	50	8·0440	6·7127	3·0523	0·9598	2·0925	68·55
	b. 163 seeds	Ignited pumice	100	8·1360	6·7895	3·0872	1·1952	1·8920	61·28
	c. Meal.....	Ignited soil	40	8·9671	7·4830	3·4025	1·0995	2·3030	67·68
3. Beans	a. 7 seeds...	Ignited soil	50	5·7830	4·5830	2·2915	0·8511	1·4404	62·86
	b. 7 seeds...	Ignited pumice	50	6·4700	5·1275	2·5637			
	c. Meal.....	Ignited soil	40	6·1750	4·8937	2·4468	0·9778	1·4690	60·04

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TABLE XV.

Substances involved in the experiment.				Total nitrogen.				Nitrogen obtained as ammonia by distillation with weak alkali.			
Organic matter.		Matrix.	Water added.	Before decom- position.	After decom- position.	Loss (or gain).		From oxalic acid in C.		From total products.	
Description.	Condition.					Actual quantity.	Per cent.	Actual quantity.	Per cent.	Actual quantity.	Per cent.
			cub. cent.	grms.	grms.	grms.		grms.		grms.	
1. Wheat	a. 171 seeds	Ignited soil	50	0·1392	0·1398	+0·0006	+0·43	0·00038	0·273	0·0429	30·83
	b. 171 seeds	Ignited pumice	100	0·1396	0·1214	0·0182	13·08	0·00002	0·014	0·0573	41·06
	c. Meal.....	Ignited soil	40	0·1709	0·1680	0·0029	1·74	0·00040	0·234	0·0197	11·49
2. Barley	a. 163 seeds	Ignited soil	50	0·1247	0·0746	0·0501	40·20	0·00055	0·441	0·0157	12·64
	b. 163 seeds	Ignited pumice	100	0·1261	0·1052	0·0209	16·62	0·00002	0·016	0·0294	23·39
	c. Meal.....	Ignited soil	40	0·1390	0·1311	0·0079	5·66	0·00039	0·280	0·0166	11·97
3. Beans	a. 7 seeds...	Ignited soil	50	0·2417	0·2107	0·0310	12·84	0·00341	1·424	0·0140	57·91
	b. 7 seeds...	Ignited pumice	50	0·2704	0·2380	0·0324	11·99	0·00242	0·895		
	c. Meal.....	Ignited soil ...	40	0·2581	0·2267	0·0314	12·16	0·00060	0·232	0·1039	40·25

The results in Tables XIII and XV are mutually confirmatory in their more general indications; and they agree in showing no tangible relation between the nitrogen-products and the varied circumstances of decomposition. In some cases there has been no, and in others very great evolution of free nitrogen—so great, indeed, that we made repeated analyses to satisfy ourselves of the truth of the result.

The physical condition of the several substances at the termination of the experiment, as also the comparative uniformity in the proportion of carbon given off (see Table XIV), might lead to the conclusion that the decomposition had proceeded about equally far in all cases; but whilst the proportion of carbon given off ranged between 60 and 70 per cent. of its original amount, that of the nitrogen varied coincidently from 0 to 40 per cent. of the original amount. Again, the proportion of the nitrogen which was retained in the mass, or absorbed in the oxalic acid in C, in such form as to be given off as ammonia on distillation after being rendered weakly alkaline, and which probably existed, therefore, in the products as ammonia, ranged from 12 to 58 per cent. of the total quantity involved in the experiment; whilst the proportion evolved from the mass during the decomposition and retained in the oxalic acid solution (C), varied from 0 to about 1·5 per cent. of the original amount. There would seem, in fact, to be no relation between the loss of carbon, the loss of nitrogen, the formation of ammonia, and the evolution of it from the mass during decomposition, on the one hand, and the circumstances of

matrix, moisture, growth, decay, &c., on the other, which can with safety be considered as exhibiting cause and consequence.

It may, however, be concluded, that under particular, and apparently rare circumstances of the decomposition of nitrogenous organic matter, there is no loss of nitrogen evolved in the free state, but that, under a wide range of circumstances, a considerable loss of nitrogen takes place; and whilst the proportion of the nitrogen taking such form that it may be driven off as ammonia on the distillation of the products with a weak alkali-solution, varied from one-eighth to more than one-half of the total, the amount evolved from the mass as ammonia during decomposition was quite inconsiderable.

Independently of the importance of these facts, considered in connection with the conditions which may be involved in an experiment on the question of the assimilation of free nitrogen by plants, it is obvious that they have a very important bearing on the practical question of the management of the manure-heap, and as such, require further investigation to ascertain the causes of the differences of result manifested, in order, if possible to control them. The results also point to the probable insignificance of the loss of nitrogen from decomposing manure in the form of ammonia, a supposed evil to which the attention of agricultural chemists has been especially directed, whilst the apparently much larger loss, as free nitrogen, has not at all been considered.

The question arises, what is the character of the action by which nitrogenous compounds are decomposed with the evolution of free nitrogen? It may be—(1) of an oxidizing character analogous to that of chlorine on ammonia; (2) of a reducing character similar to that of many substances upon the oxygen compounds of nitrogen; (3) these two actions may operate in succession the one to the other.

The nitrogen in the organic substances submitted to decomposition, doubtless existed in a condition more analogous to a hydrogen, than to an oxygen-compound of it; and the researches of Hofmann would lead us to suppose that the nitrogen-compounds in question were of the ammonia class. They are more difficult to oxidize into nitric acid than is ammonia, but their transition into ammonia is extremely easy; and, since ammonias yield free nitrogen under the influence of oxidising forces, it may be inferred that it has been under the influence of such forces that the nitrogen has been set free in the experiments recorded. Pelouze

has remarked* that nitrates are converted into ammonia in contact with decomposing organic matter; and experiments of our own have shown that, during the decomposition of organic matters in contact with nitrates, free nitrogen is not evolved.

The experiments next referred to bear upon these points.

After several qualitative experiments, on a smaller scale, about half a pound of a mixture of wheat, barley, and beans, was put into the long narrow glass vessel of about 500 cub. cents. capacity (plate I, fig. 7), which was then filled with well-boiled water, and closed with a cork, through which two glass tubes (*a* and *b*) passed, the ends of which were fitted with caoutchouc tubing, for closing with pinchcocks, or connexion with the Torricellian exhauster. The vessel having been so connected with the exhauster for several hours, to remove the gaseous nitrogen from the seeds, it was then inverted in mercury, the tube *b* opened under that fluid, and the whole placed in sunlight to favour decomposition. This was done on August 28 (1858); the seed soon swelled, and on August 30 well-marked decomposition had set in. On September 13, the vessel was about two-thirds full of gas, and so much water was displaced, that part of the seed was above the remaining water, in the gas, which commenced bubbling out through the tube *b*. The arrangement so remained until October 5, when 400 cub. cents. of gas were collected, of which the percentage composition was as follows:—

	Carbonic Acid.	Hydrogen.	Nitrogen.
Experiment 1	64·87	34·83	0·30
Experiment 2	64·54	35·46	traces

The quantity of gas evolved points to the extent of the decomposition; the amount of carbonic acid and hydrogen shows how great must have been the reducing force exerted; and the small quantity of nitrogen, which was probably due to accident, indicates that free nitrogen was not a product of the action.

The vessel was again filled with boiled water, again connected for some time with the Torricellian exhauster, and again placed in its former position in the sunlight. On November 17, only a few bubbles of gas having been evolved, the vessel was removed into a room, the temperature of which varied from a few degrees above the freezing point to about 24° C. On December 12, the gas

* Comptes Rendus, xliv, p. 118.

collected without exhaustion measured only 6·1 cub. cents., of which 4·6 were absorbed by potassa, and the remainder was combustible and contained no appreciable amount of free nitrogen.

Five grammes of nitrate of potassa were now put into the vessel (with the same organic matter), and it was replaced in the room as before. On May 25, 1859, the vessel having been heated up to 30° C. several times during the interval, only 4 cub. cent. of gas were collected, one-fourth of which was carbonic acid, and the remainder combustible.

The vessel was now placed in sunlight again, but up to the middle of June no more gas was evolved. The fluid still contained nitrate. The vessel was now half-filled with oxygen; but after ten days not one-fourth of the supplied oxygen had been consumed.

The total gas being removed, oxygen was conducted into the vessel until the greater part of the fluid was driven out, leaving the partly decomposed seeds in an atmosphere of this gas. The apparatus so arranged was placed in the sunlight, and remained there during some very warm weather, when, on July 12, the gas collected contained in 100 parts—carbonic acid 20, oxygen 79, nitrogen 1; but as, by accident, a little air had been admitted into the vessel, the small quantity of nitrogen found may be attributed to it.

On removal, the beans were found to possess much of their original firmness; but the other seeds, though retaining their form, were softer, and had evidently undergone more complete decomposition. Very little odour was emitted, and it was not unpleasant.

It is obvious that there had been no evolution of free nitrogen during the long period that these matters had been submitted to decomposition under the conditions described.

Experiments were also made upon the products of the decomposition of organic matter in the first stages of the process. The decomposition took place in water, in the tall glass vessels already described. In Table XVI are given the amounts, and the composition, of the gas obtained in a few out of numerous experiments; and, for comparison, some of the results already referred to are included.

TABLE XVI.

Description of Organic matter subjected to decomposition	Total gas collected.	Composition of the gas, per cent.		
		Carbonic acid.	Hydrogen.	Nitrogen.
	cub. cents.			
<i>a.</i> Wheat, Barley, and Bean seed ..	400	64·87	34·83	0·30
<i>b.</i> Turnip plant; root with leaves.	166·2	64·54	35·46	traces.
<i>c.</i> Turnip plant; root with leaves.	162·2	76·23	22·91	0·87
<i>d.</i> Turnip plant; root with leaves.	123·6	68·83	23·93	7·24
<i>e.</i> Turnip plant; root with leaves.	41·2	68·06	25·63	6·31
		67·52	25·43	7·05
		64·95	14·66	20·39

Experiment (*a*) is the one described above. In all the other cases, about two ounces of plant were operated upon. The experiment commenced on August 29, and was stopped on October 5, when the structure of the plant was almost entirely destroyed, there remained only a mass of decomposed matter at the bottom of the vessels, and the evolution of the gas had entirely ceased. The plant (*b*) was exhausted of its gas before exposure; and, under these circumstances, a very small quantity of free nitrogen was found at the termination of the experiment, whilst plants *c*, *d*, and *e*, which were not so exhausted, gave a larger amount of free nitrogen; and, although in experiment (*e*) the percentage of nitrogen was much higher than in the other cases, the total quantity of gas was much less, and the actual quantity of nitrogen was nearly the same in the several cases, leading to the supposition that it existed within the plant at the commencement of the experiment.

The result is, then, that, in the absence of free oxygen, no free nitrogen was evolved from the decomposing nitrogenous compounds, and it may be inferred that the loss of nitrogen indicated in Tables XIII and XV was the result of an oxidising process.

Some experiments were also made in which the plan was to place nitrogenous organic matter in an atmosphere of pure oxygen, and to afford a constant supply of the gas as it was consumed and the carbonic acid produced was absorbed by a solution of caustic potass. The results obtained indicated an evolution of free nitrogen; but owing to the difficulty of getting the requisite apparatus absolutely air-tight, they were open to some exception, and the investigation was, for the time, abandoned.

It is obvious that the results recorded point to a serious difficulty in the conduct of experiments on the question of the assimilation of free nitrogen by plants; for although, as Boussingault has shown, there may be no loss of nitrogen during germination, yet, during the entire period of growth of a plant, portions of nitrogenous organic matter may be subject to decomposition under conditions favourable to the evolution of free nitrogen.

The following experimental results are interesting in connexion with this point. Eight seeds, each, of wheat, barley, and oats, were respectively sown in prepared soil, precisely as in the experiments on assimilation. The three pots were placed under a glass shade, 16 inches in diameter, which rested in the groove of a stoneware lute-vessel containing sulphuric acid. About 500 cub. cent. of distilled water were added to each soil at the commencement, but no carbonic acid was supplied beyond that which might be contained in the water. The pots, enclosed as described, were then exposed to the diffused light of the laboratory, but without the access of direct sunlight. In a few days, all came up, and grew very rapidly in height, but with little development and expansion of leaf; all the plants being tall, slender, and delicate, and having the pale green colour common to plants in deficient sunlight. In other experiments, plants in this condition, when removed into sunlight, ceased the predominant upward tendency of growth, the long delicate first leaves lost their vitality, numerous shorter and broader ones were formed, and the stems became thicker and more dense.

The experiment under consideration commenced on May 17, 1858, and continued until June 10 (24 days), when the plants had ceased to grow. All the plants were much alike; ranging from 7 to 12 inches in height, each having three leaves, two lateral ones from 8 to 12 inches long, and a shorter, unrolled terminal one. The stems of several were so slender and delicate as to fall over. The roots were found to consist of short fibriles, with divaricated branchlets, seldom extending more than 2 or 3 inches from the seed. Table XVII gives the analytical results of the experiment:—

TABLE XVII.

Particulars of seed sown.			Particulars of the products.						
	Dry matter.	Nitro-gen.	Dry vegetable matter.			Nitrogen.			
			Stems.	Roots.	Total.	In stems & roots.	In soil & pot.	In total products.	Gain or loss.
	gram.	gram.	gram.	gram.	gram.	gram.	gram.	gram.	gram.
Wheat	0·4077	·00790	·320	·140	·460	·00697	·0012	·00817	+ ·00027
Barley	0·3234	·00573	·290	·160	·450	·00570	traces	·00570	- ·00003
Oats..	0·2900	·00640	·355	·060	·415	·00640	traces	·00640	+ traces

Lest the accuracy of the result in regard to the nitrogen should be endangered, the roots were but slightly washed, and retained particles of soil, in consequence of which the weights given for their dry matter are a little too high. There was, however, evidently a slight gain of dry matter, the carbon of which was doubtless due to carbonic acid in the distilled water which had been supplied. The rapid growth, its short duration, the limited distribution of the roots, and the fact that no water was added during growth, which might distribute easily transportable matters, are conditions which are all consistent with the almost total absence of nitrogen in the soil.

The last column in the Table (XVII) shows that in these experiments no free nitrogen was given off during the process of germination and growth; at least the assumption that free nitrogen was evolved implies the still more improbable one that an exactly equal amount of free nitrogen was assimilated.

It would appear, therefore, that in an experiment on the question of the assimilation of free nitrogen by plants, little fear of loss of nitrogen, due to the decomposition of nitrogenous organic matter, need be entertained, so long as that matter is subjected to the ordinary process of germination, and exhaustion to supply materials for growth; though there may be considerable danger of such loss if nitrogenous organic matter were used as manure, or in case of the decomposition of sterile seeds, dead leaves, old roots, or nitrogenous excretions. In judging, according to the conditions of the assimilation experiment, of the probable extent of such evolution, it should be borne in mind that, in the cases where the large evolution of free nitrogen took

place in the experiments on decomposition, the organic matters were subjected to the action for a period of about six months, during which time their organic form was generally entirely destroyed, and they lost nearly three-fourths of their carbon.

One more collateral point requires to be noticed before entering upon the consideration of the direct experiments on the question of assimilation.

Results quoted above leave no doubt of the evolution of hydrogen during the decomposition of organic matter, and Mulder,* and others, have attached much importance to the mutual action of nascent hydrogen so evolved, and gaseous nitrogen, as a source of ammonia. That nascent hydrogen may, under certain circumstances, combine with gaseous nitrogen, has long been admitted; but the view that such combination takes place when hydrogen is evolved from decomposing organic matter, requires confirmation. If but a small part of the hydrogen evolved in the decomposition of organic matter in peat-bogs, in cesspools, in stagnant water, in the annual deposits of leaves in the extensive forests in different parts of the world, decaying under the influence of moisture and confined air between the succeeding layers, as well as in many soils, were to combine with free nitrogen, how enormous would be the amount of ammonia formed in this way—the amount due alone to the decomposition of the exuberant growth of former geological periods would be incalculable.

The results which have been given show, that in the decomposition of nitrogenous organic matter, both free hydrogen and free nitrogen may be given off; and the assumption that nascent hydrogen under such circumstances unites with the nitrogen of the air, implies that it was capable of uniting with free nitrogen under circumstances in which its affinities were not sufficiently powerful to prevent nitrogen-compounds, very similar to ammonias, from giving up nitrogen in the free state; and also that it could act upon ordinary nitrogen when it could not do so upon the nascent nitrogen of the decomposing nitrogenous body. Or, if it did act upon the latter, there would either be no free nitrogen evolved, or if there were, it would show that less nascent nitrogen had been converted into ammonia than had had been liberated from its combinations, the result of which would be a loss, and

* Chemistry of Vegetable and Animal Physiology, pp. 111-114, 149-152, &c.

not a gain of combined nitrogen. But the fact is that both free hydrogen and free nitrogen are given off.

It would seem from the above considerations, that there need be little apprehension of error in the results of experiments on the question of assimilation, arising from an unaccounted supply to the plants of ammonia formed under the influence of nascent hydrogen given off in any decomposition of the organic matter involved in the experiment.

Having described the arrangement adopted in our experiments on the question of the assimilation of free nitrogen by plants, and discussed some collateral points, particularly those which relate to the possible sources of gain or loss of combined nitrogen to the plants under experiment, it only remains further to preface the statement of the experimental results obtained, by considering what are the most probable conditions for the assimilation of free nitrogen, provided it can take place at all?

The questions arise—whether such assimilation would be most likely to take place, when the plant had no other supply of combined nitrogen than that in the seed sown, when supplied with a limited amount of combined nitrogen, or with an excess of combined nitrogen? And again—whether at an early stage of growth, at the most active stage, or when the plant is approaching maturity? Combinations of these several circumstances might give a number of special conditions, in perhaps but a few of which assimilation of free nitrogen could take place, if in any.

It would hardly be supposed that free nitrogen would be assimilated when an excess of combined nitrogen was at the disposal of the plant. It is obvious, however, that a wide range of conditions would be experimentally provided, if, in some instances plants were supplied with no more combined nitrogen than that contained in the seed sown, in others they were brought to a given stage and activity of growth by means of limited extraneous supplies of combined nitrogen, and in others the supply of combined nitrogen were still more liberal. It has been sought to provide these several conditions in the experiments under consideration. In either case supposed above, the plant will, at a certain stage, or at certain stages, as the case may be, have an excess of combined nitrogen at its disposal in relation to its immediate wants, and will pass from this point through all stages in regard to supply, to that in which combined nitrogen is in defect. If, how-

ever, the plant cannot assimilate free nitrogen unless it has attained a certain vigour or stage of growth, it may, perhaps, be alleged, in case of no assimilation taking place, that the supply of combined nitrogen has been insufficient to bring it to the supposed point; but if, on the contrary, the assimilation can take place at all periods of growth, and in the absence of all extraneous sources of combined nitrogen, the solution of the question becomes much more easy. Indeed, even in an experiment without any extraneous supply of combined nitrogen, all the conditions with regard simply to the relative quantity of combined nitrogen, are provided. Thus, when the seed is first sown, it contains within itself an excess of combined nitrogen so far as the immediate wants of the plant are concerned, and it will gradually pass to the point at which all available combined nitrogen has been appropriated, and only free nitrogen is presented to it. A negative result under such circumstances even, would afford evidence against assimilation of free nitrogen under a wide range of conditions; and a similar result with various amounts of combined nitrogen supplied, would be conclusive as to a still wider range of circumstances of growth.

The last point for consideration is as to the selection of the plants to submit to the adopted conditions of the experiment. It was sought—(1) to have such as would be adapted to the conditions of temperature, moisture, &c., to which they were to be subjected; (2) to have such as were of importance in an agricultural point of view; (3) to acquire the means of studying any difference, in reference to the point in question, between plants belonging, respectively, to the two great natural orders, the Graminaceæ and the Leguminosæ, which, in some points of view, appear to differ so widely in their demands upon combined nitrogen provided within the soil; (4) to take such as had already been experimented upon, with such conflicting results, by M. Boussingault and M. G. Ville.

Experiments in which the Plants had no other supply of Combined Nitrogen than that contained in the Seed sown.

Table XVIII, p. 172, gives, at one view, a summary of the numerical results obtained under this head; see also figs. 1—6, plate III, which are reduced from careful drawings of six out of the nine Graminaceæ experimented upon, and illustrate the

character and extent of growth attained under the conditions in question.

After so full a discussion of the circumstances under which the results were obtained, but little more is needed in pointing out their bearings upon the question at issue, than to direct attention to the column showing the gain or loss in each experiment.

The differences between the results obtained with soil and with pumice as matrix, in 1857, are not such as can be attributed in any way to the difference of matrix; indeed, the parallel experiments with the two descriptions of soil may be considered as duplicates.

Graminaceous Plants.

It is seen that the largest gain of nitrogen in the three experiments with Graminaceæ, in 1857, was 0·0026 gramme. In regard to the fact that there was generally a somewhat larger gain of nitrogen in some of the experiments in 1857 than in those in 1858, it may be observed that, soon after watering with the fluid drawn off from the surface of the slates used in the former year as lute-vessels, the soils in one or two cases became more or less covered with a slight coating of green matter. And nearly all the slates were found at the end of the experiment to have a similar coating beneath the pans in which the pots stood; whilst, in the experiments of 1858, when glazed earthenware lute-vessels were employed, no such phenomenon was observed. Keeping in view this fact, and considering that so small an amount of nitrogen had to be determined in so large an amount of soil (0·003 gramme or less of nitrogen in about 1,500 grammes of soil), it seems more than questionable whether the small gain above quoted, should not be attributed to errors of experiment or analysis. In fact, we can but conclude, that under the circumstances of growth of the Graminaceous plants to which Table XVIII relates, there has been no assimilation of free nitrogen. There is, indeed, in no case more nitrogen in the plant itself than in the original seed, the gain appearing only when that in the soil and pot is taken into account.

The experiments on germination and growth referred to at pp. 166–7, showed how completely the plants could appropriate the nitrogen of the seed, leaving only traces in the soil; and the experiments on decomposition showed how complete was the action coincident with the passage of any large proportion of the

TABLE XVIII.

Results of Experiments to determine whether Plants supplied with no combined Nitrogen beyond that in the Seed sown, assimilate free Nitrogen.

Years.	Plants.†	Number of seeds.	Period of growth.	Dry substance.		Nitrogen.										Per cent. in dry produce.
				Grammes.		Grammes.										
				In seed.	In plants produced.	In seed.	In total plant.	In soil.	In pot.	In condensed water.	In total products.	Gain or loss.				
Graminaeae.																
1857.....	{ Wheat (1) Barley (2) Barley (3)	6	5	May 16—Oct. 3.....	0.3065†	1.412	0.080	0.072	traces	traces	traces	-0.072	-0.008	90.0	0.51	
		6	6	May 20—Aug. 24.....	0.2698	0.810	0.056	0.047	0.024	0.001	0.016	-0.072	+0.016	83.9	0.58	
		6	6	May 20—Aug. 25.....	0.2698	0.925	0.056	0.045	0.030	0.007	0.026	-0.052	+0.026	80.4	0.48	
1858.....	{ Wheat (4) Barley (5) Oats (6)	8	8	April 27—Oct. 25.....	0.4035	1.740	0.078	0.056	0.025	traces	traces	-0.081	+0.003	71.8	0.32	
		8	6	April 27—Aug. 18.....	0.3221	0.560	0.057	0.031	0.027	traces	traces	-0.058	+0.001	54.4	0.54	
		8	8	April 27—July 13.....	0.2858	1.148	0.063	0.042	0.011	0.003	0.003	-0.056	-0.007	66.7	0.36	
1858, A*.....	{ Wheat ... Barley ... Oats	8	7	June 11—Nov. 6.....	0.4031	1.060	0.078	0.041	0.033	0.004	0.004	-0.078	0.000	52.6	0.38	
		8	8	June 11—Nov. 6.....	0.3240	0.710	0.057	0.038	0.021	0.004	0.004	-0.063	-0.001	59.4	0.55	
		8	7	June 11—Nov. 6.....	0.2879	0.690	0.064	0.038	0.021	0.004	0.004	-0.063	-0.001	59.4	0.55	
Leguminosae.																
1857.....	Bean	2	2	May 16—July 5.....	1.4984	7.028	0.0796	0.029	0.016	0.016	0.016	-0.791	-0.005	79.0	0.89	
		3	3	June 11—Aug. 23.....	1.4820	4.875	0.0750	0.035	0.016	0.016	0.016	-0.757	+0.007	98.0	1.51	
1858.....	{ Pea	3	3	June 5—Aug. 24.....	0.5405	0.970	0.188	0.102	0.056	0.009	-0.167	-0.021	54.3	1.05		
		3	3	June 5—Aug. 24.....	0.5405	0.970	0.188	0.102	0.056	0.009	-0.167	-0.021	54.3	1.05		
Other Plants.																
1858.....	Buckwheat.....	24	13	Aug. 20—Oct. 28.....		0.450	0.0200	0.070	0.108	0.004	0.004	-0.182	-0.018	35.0	1.56	

* These experiments were conducted in the apparatus of M. G. V. 111e.

† The numbers given in brackets refer to those of the figures of the respective plants given in Plate III.

‡ The percentage of dry matter in the seed was not determined in this case; it is therefore assumed to be the same as in the wheat used in 1858, from which it would certainly not differ at all materially.

nitrogen of the substance into the soluble state of ammonia. It would appear probable, therefore, that at least a part of the nitrogen found in the soil had never been in actual connexion with the plant; and it must be admitted, that a gain in the total products, without a gain in the plant itself, would, under the conditions in question, be unsatisfactory evidence of the assimilation of free nitrogen.

The results obtained with Graminaceæ in 1858, when some sources of error which the experience of the previous year had suggested had been eliminated, point, without exception, to the non-assimilation of free nitrogen. The process of cell formation has gone on; carbonic acid has been decomposed, and carbon and the elements of water assimilated; the nitrogenous compounds of the older cells have been withdrawn for the requirements of new ones; the roots, requiring the smallest amount of nitrogen, were extraordinarily developed; and all stages of growth have been passed, to the formation of glumes, pales, and awns, for seed, the other organic constituents increasing with one constant amount of combined nitrogen until its percentage in the vegetable matter was far below the usual amount. Throughout these phases, water saturated with free nitrogen has constantly been in contact with the contents of the cells and the cell-walls; the delicate membrane of the newly-forming cell, stunted in its development for want of assimilable nitrogen, has been saturated with water, itself saturated with free nitrogen; and such are the laws in accordance with which the absorption of gases, and the transmission of liquids through membranes take place, that the instant a part of the nitrogen became assimilated the equilibrium would be restored and the liquid resaturated, circumstances which would seem highly favourable to the assimilation of free nitrogen, provided it could take place at all.

In the experiments of 1857, several of the cereal plants developed a very large proportion of root, but fearing the double risk of losing the entire results in analysing the root and the rest of the plant separately, they were thoroughly mixed, and the mixture carefully divided, so as to provide confirmatory evidence if successful, or a duplicate in case of accident. The development was, however, so marked in the cereals of 1858, that the root was in several cases analysed separately. Table XIX shows the results obtained :—

TABLE XIX.

Plants.* (1858).	Dry matter.				Nitrogen.			
	In stems, &c.	In roots.	In total plant.	Per cent. of total in roots.	In stems, &c.	In roots.	In total plant.	Per cent. of total in roots.
	grm.	grm.	grm.		grm.	grm.	grm.	
Wheat (4) .	0·890	0·850	1·740	48·85	0·0039	0·0017	0·0056	30·36
Barley (5) .	0·400	0·160	0·560	28·57	0·0027	0·0004	0·0031	12·90
Oats (6) ..	0·798	0·350	1·148	30·49	0·0040	0·0002	0·0042	4·76

The large proportion of root, and its small proportion of nitrogen are equally remarkable. Whether the great development of root were due to the process of cell formation in it requiring less of the nitrogenous proto-plasmic compound, or to the withdrawal, by the fluid in which the roots floated, of the nitrogenous constituents from the old cells to form new proto-plasma for the more active cells, is a question of considerable interest in a physiological point of view. The fact that the roots gave off very few branches into the soil, but immediately on reaching the water in the pan exhibited such a remarkable development, is in favour of the inference that the water afforded the necessary conditions.

At any rate, this great development of the part of the plant requiring a minimum of nitrogen indicates an inability to assimilate free nitrogen within the range of growth possible when no combined nitrogen is provided beyond that in the seed sown; and it shows the great tenacity of growth, and activity of vital force, long after the organism began to require more available nitrogen—conditions apparently very favourable to the assimilation of free nitrogen; yet such has not been the result.

The Graminaceæ referred to in Table XVIII. under the title "1858 A," which were grown in M. G. Ville's case, give results perfectly accordant with the rest; but being sown later, and their period of growth being shorter, there was neither so great a development of root nor so much total vegetable matter produced. Unfortunately, the barley of the series was lost by the giving way of the tube in combustion; so that, in its case, we can only give the amount of dry matter produced; but, comparing the

* The numbers given in brackets refer to those of the figures of the respective plants given in plate III.

results on this point with those in the other cases, there is no reason to believe that those in regard to the nitrogen would have been different.

It will be remembered that M. Boussingault and M. G. Ville obtained most discordant results in experimenting with Graminaceæ, and it will be seen that our own results, from nine experiments with such plants, go entirely to confirm those of M. Boussingault.

Leguminous Plants.

It has been shown in section 1 of this paper, that, under equal circumstances of soil and season, Leguminous crops yield two, three, or more times as much nitrogen per acre, as Graminaceous ones; that, nevertheless, the latter are very characteristically benefited by the direct use of ammoniacal manures, whilst the former are not so; and again, that a much larger Graminaceous crop is obtained after the growth and removal of a highly nitrogenous Leguminous one, than after another Graminaceous crop. Experiments such as those now under consideration, can obviously bear upon a few only of the circumstances which may be connected with the differences referred to. They do, however, bear upon the questions—(1) whether in the growth of the Graminaceæ, there is a decomposition of nitrogenous compounds and an evolution of free nitrogen? or, (2) whether the Leguminosæ may assimilate free nitrogen, and thus not only allow the resources of the soil to accumulate, but leave within it, in roots and other vegetable *débris*, an additional quantity of combined nitrogen?

The facts already considered do not indicate an evolution of free nitrogen from the nitrogenous compounds of Graminaceous plants during growth; and others, to which we shall presently refer, afford confirmatory evidence in the same direction.

With regard to the second point, as the growth of the Leguminous plants under the experimental conditions was generally not so healthy as that of the Graminaceæ, it may, perhaps, be maintained, that a negative result with them was not so conclusive as with the latter. In 1857, several experiments with beans were commenced, but they grew well in only one of the shades. These, however (especially one plant out of the two in the same pot), progressed remarkably well for 10 weeks, during which time the amount of dry matter, and consequently of carbon, increased

about five-fold; more than three-fourths of the nitrogen of the seed was appropriated, and the plants probably ceased to grow only when the remainder became so distributed in the soil as not to be available. The beans and peas of 1858 did not grow so satisfactorily as the beans of 1857 last referred to; yet the beans (of 1858) gave more than three times as much organic matter in the produce as was contained in the seed, and appropriated even a much larger proportion of the nitrogen of the seed than did those of 1857. The result with the peas was not so satisfactory, owing to the less healthy and more limited character of the growth. It may be added that none of these Leguminous plants arrived at the stage of flowering.

The result is, however, that active growth has taken place—that the process of cell formation, with the decomposition of carbonic acid and fixation of carbon, has gone forward with a deficient supply of combined nitrogen, and in the immediate presence of free nitrogen, both gaseous and dissolved—in fact, that the plants have been subjected to a considerable range of the conditions which appear, *à priori*, to be favourable to the assimilation of free nitrogen, yet none has been assimilated by these Leguminous plants any more than by the Gramineous ones.

It is a fact observed in agriculture, that manures rich in organic matter, frequently favour the growth of Leguminous crops; and without discussing the question whether they act as a source of carbonic acid merely, or of carbon-compounds of a more complicated character, attention may be called to the fact, that, in the experiments now under consideration, the vital forces were sufficiently energetic to perform the function of cell development and multiplication, from carbonic acid as the source of carbon, and yet they were incapable of effecting the appropriation of free nitrogen.

Buckwheat.

In the experiment with buckwheat, most of the seed sown came up, but about half of the plants lived only for a few days. The remainder went through all the stages of development to flowering, but the amount of growth was nevertheless very limited, the quantity of dry matter produced being less than that in the seed sown, and the nitrogen in the plants little more than one-third that in the seed. It will be seen further on, however, that on the addition of an amount of ammonia very small in its contents of

nitrogen compared with the seed, to plants in a precisely similar condition to those now under consideration, the increase of growth was extremely marked.

Reference to the column in Table XVIII. giving the gain or loss of nitrogen, will show that, under the conditions of growth described, there was no gain of nitrogen by the buckwheat, but on the contrary a loss of nearly 2 milligrammes, doubtless due to the evolution of free nitrogen in the decomposition of some of the plants that died.

Various experimenters, from the time of De Saussure, have entertained the idea of the probability of the decomposition of nitrogenous compounds, and the evolution of free nitrogen, during the growth of plants. We have been engaged in following up the subject in a more direct manner, but attention may here be called to the incidental bearing upon it of the results above considered.

With no less force than they point to the non-assimilation of free nitrogen, do they show that there has been no evolution of free nitrogen from the nitrogenous compounds of the growing plant. At all events, from the constancy of the amount of combined nitrogen maintained in relation to that supplied, the assumption that an evolution has taken place, implies the still more improbable one, that there has been an exactly compensating assimilation. But, when supplied with an insufficient quantity of nitrogenous matter, the vegetable organism might not decompose any of that matter ; and yet, with an excess of combined nitrogen, decomposition might take place. We shall, therefore, refer to this question again, in connexion with the results obtained when an extraneous supply of combined nitrogen was provided to the plants.

Experiments in which the Plants had a known supply of Combined Nitrogen beyond that contained in the Seed sown.

Although in the experiments already considered, the extent of growth was very limited, some of the cereals did indeed produce glumes and pales for seed ; but none attained to the formation and ripening of the seeds themselves. It remains to consider, therefore, what may take place with more active and vigorous growth, and at a later stage of development.

We have suggested the improbability of an assimilation of free nitrogen taking place in the presence of an abundance of combined nitrogen; but if active, healthy, and vigorous growth are favourable to the assimilation of free nitrogen, and these conditions can only be attained by keeping within the reach of the plant an excess of combined nitrogen, we should have to conclude that free nitrogen could be taken up in the presence of, and in preference to, combined nitrogen; *à priori* conclusions on points involving vital phenomena are, however, obviously unsafe.

It is true, we have pointed out the improbability of an assimilation of free nitrogen in the presence of an excess of combined nitrogen, only so far as the vital process of the vegetable cell is concerned. But, the more active the growth, the greater must be the amount of newly-formed carbon-matter capable of consuming oxygen when the plant is removed from the influence of sunlight into the dark. That is, the more vigorous the growth in the sunlight, the greater may be the reducing power of the plant in the dark; and the greater this reducing power, the more nearly will the tendency of the forces approximate to an evolution of hydrogen, which, in the presence of free nitrogen dissolved in the fluids of the cell, may tend to form ammonia, to be, on the return of sunlight, appropriated by the plant. In connexion with this point, it may be mentioned that in our investigation of the gases given off by plants under various circumstances, we have had an evolution of oxygen one day as a coincident of growth, and an evolution of hydrogen the next, as the result of decomposition.

The results of the experiments in which the plants were supplied with combined nitrogen beyond that in the seed sown, are given in Table XX, p. 179; and the figures 7—12, plate III, show the character and extent of development of six Gramineous plants with extraneous supply of combined nitrogen, corresponding to the six above them without such supply; and the vigour and extent of growth attained, may be taken as sufficient proof that the conditions of soil, atmosphere, temperature, &c., were consistent with active and healthy growth. As already explained, the combined nitrogen was supplied by means of dilute solutions of sulphate of ammonia.

It will be instructive to consider the results to which Table XX refers, in regard—(1) to the actual gain or loss of nitrogen; (2) to the physiological evidence afforded during growth; (3) their indications as compared with the results obtained when there

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TABLE XX.

Results of Experiments to determine whether Plants supplied with combined Nitrogen beyond that in the Seed sown, assimilate free Nitrogen.

Years.	Plants.†	Number of seeds.	Period of growth.	Dry substance.			Nitrogen.												
				Grammes.			Grammes.							In plants to 100 in seed and manure.	Per cent in dry produce.	In produce per seed with ammonia, to that without it taken as 1.			
				In seed.	In plants produced.	In produce to that sown taken as 1.	Total supplied.	In total plant.	In soil.	In pot.	In condensed water.	In total produce.	Gain or loss.						
Graminacæ.																			
{ 1857... }	Wheat (7)...	3	2	May 16—Oct. 2 ...	0.1586†	6.835	44.50	12.10	-0.040	-0.289	-0.329	-0.241	-0.133	-0.009	-0.383	+0.054	73.3	0.35	8.36
	Wheat (8)...	3	3	May 16—Sept. 20 ...	0.1543†	8.822	24.79	4.51	-0.040	-0.389	-0.329	-0.213	-0.102	-0.016	-0.381	+0.002	64.7	0.55	4.93
	Barley (8)...	4	4	May 20—Oct. 8 ...	0.1803	3.034	16.83	7.49	-0.037	-0.289	-0.326	-0.154	0.171	-0.003	-0.328	+0.002	47.2	0.51	6.58
	Barley (9)...	4	4	May 20—Sept. 24 ...	0.1818	4.401	24.18	7.13	-0.037	-0.231	-0.268	-0.145	-0.171	-0.021	-0.337	+0.069	54.1	0.33	4.83
{ 1858... }	Wheat (10)...	4	4	April 27—Oct. 26 ...	0.204	7.31	35.83	8.40	-0.040	-0.508	-0.548	-0.398	-0.138	traces	-0.536	-0.012	72.6	0.54	14.21
	Barley (11)...	4	2	April 27—Oct. 26 ...	0.158	5.47	34.62	29.31	-0.028	-0.468	-0.496	-0.349	-0.113	-0.002	-0.464	-0.032	70.4	0.64	34.21
	Oats (12) ...	4	3	April 27—July 30 ...	0.145	1.204	8.31	2.80	-0.032	-0.280	-0.312	-0.125	-0.040	-0.051	-0.216	-0.096	40.1	1.04	7.92
	Wheat (13)...	4	4	June 29—Dec. 9 ...	0.206	3.82	18.54	6.31	-0.040	-0.228	-0.268	-0.181	-0.092	-0.001	-0.274	+0.006	67.5	0.47	7.79
{ 1858, A* }	Barley	4	3	June 29—Dec. 9 ...	0.162	2.98	18.40	18.40	-0.029	-0.228	-0.257	-0.160	-0.081	-0.001	-0.242	-0.015	62.3	0.54	
	Oats	4	2	June 29—Dec. 9 ...	0.144	1.28	8.89	6.49	-0.032	-0.228	-0.260	-0.146	-0.051	-0.001	-0.198	-0.062	56.1	1.14	13.52
Leguminosæ.																			
{ 358... }	Pea	3	3	April 27—Aug. 24 ...	0.539	1.01	1.87		-0.187	-0.040	-0.227	-0.114	-0.086	-0.011	-0.211	-0.016	50.2	1.13	
	Clover			June 6—Oct. 26 ...		4.20			-0.284	-0.428	-0.712	-0.313	-0.339	-0.013	-0.665	-0.047	44.0		
358, A*	Bean	3	3	June 29—Dec. 9 ...	1.094	4.80	3.93		-0.523	-0.188	-0.711	-0.401	-0.253	-0.001	-0.655	-0.056	56.4	0.93	
Other Plants.																			
858.....	Buckwheat.	42	24	Aug. 20—Nov. 22 ...	0.850	1.97	2.32	2.37	-0.200	-0.108	-0.308	-0.183	-0.101	-0.008	-0.292	-0.016	59.4	0.92	1.41

* These experiments were conducted in the apparatus of M. G. Ville.

† The numbers given in brackets refer to those of the figures of the respective plants given in Plate III.

‡ The percentage of dry matter in the seed was not determined in these two cases; it is therefore assumed to be the same as in the wheat used in 1858, from which it would certainly not differ at all materially.

was no other supply of combined nitrogen beyond that in the seed sown.

As in the experiments without, so also in those with a supply of combined nitrogen beyond that in the seed sown, there is generally a slight gain of nitrogen in those of 1857, but almost invariably a loss rather than a gain in those of 1858, when glazed stone-ware, instead of slate lute-vessels were employed; and considering the possible source of gain when slates were used, the whole of the results can be interpreted but in one way.

The slight loss of nitrogen in the experiments of 1858 is easily accounted for. The general character of growth of the peas, clover, and beans, under the experimental conditions, as already referred to, taken in connection with the evidence adduced as to the loss of nitrogen during the decomposition of nitrogenous organic matter, sufficiently explains the loss in their case. The loss in the cases of the wheat, barley, oats, and buckwheat (1858), would not be so easily explained, had not the combined nitrogen in the drainage-water remaining at the end of the experiment been determined separately; and the fact that a notable quantity of ammonia was found in the condensed water, only where there was a *loss* of combined nitrogen in the experiment, would lead to the inference, that both phenomena were due to the same cause.

The condensed water showing the amounts of combined nitrogen recorded in the table, was that collected during the last four weeks of growth; and during this period the high temperature, and the advanced stage of growth of the plants, were favourable to the evaporation of ammoniacal water. Owing to the comparatively low temperature of the shade, a large proportion of the vapour would condense on its interior, and finally be collected in the bottle O; but a certain quantity of that present in the atmosphere of the apparatus, during the passage of the air through it, would be borne forward into the sulphuric acid in the bulb-apparatus M, and thus occasion a loss of combined nitrogen. The reason of the greater loss in both the experiments with oats, than in those of the other cereals, is not perfectly obvious; but in connection with this point it may be mentioned that, in one case more particularly, they ripened at a much warmer period of the season, and becoming much drier in stem and leaf, might be more liable to evolve ammonia.

It is, however, not enough to consider the actual gain or loss of nitrogen merely, but it is desirable to take into account the total

quantity of nitrogen at the disposal of the plants, and also the amount and character of growth, under the different conditions, or, in other words, the physiological evidence.

We have already called attention to the fact of the very great increase of growth when a supply of combined nitrogen, extraneous to that of the seed sown, was provided. This is strikingly illustrated by the figures in the columns of Table XX, which show the amounts respectively, of dry substance and of nitrogen, in the final produce per seed with ammonia to that without it taken as 1; but the indications manifested from the earliest periods of growth require to be briefly noticed.

When they first came up, all the plants looked green and vigorous, indicating the presence of all conditions essential to healthy growth; and there can be little doubt that, at that time, they were supplied with an excess of combined nitrogen in relation to their immediate wants. After some days, varying with the nature of the plants, they began to assume a lighter green or pale yellow tint, indicative of a want of combined nitrogen. It has been already pointed out how favourable, probably, would be the condition for the assimilation of free nitrogen, when the plant was passing from the state in which it had an excess to that in which it had a deficiency of combined nitrogen for the demands of growth. The vigorous development of the plants grown under the same conditions as the other experimental plants, but in garden soil (fig. 13, plate 3), indicates that the conditions of atmosphere, &c., provided, were not at fault. If a supply of combined nitrogen alone be deficient when the plants show the declining vigour above described, they will, on its addition, resume their healthy green colour. Or, if combined nitrogen be added before the plants decline, it will prevent them assuming the pale green or yellow colour. Both these expedients have been adopted; and so far as the cereals, buckwheat, and clover are concerned, each has yielded a result indicating that combined nitrogen alone was needed for healthy growth.

The plants to which ammonia was given in 1857, were allowed to suffer more before they received it, than those of 1858; yet in thirty-six hours after its addition to the soil, in amount not exceeding 1.5 milligramme of nitrogen to each plant, they began manifestly to improve, and in two or three days the effect was quite marked; but at the termination of periods varying from nine to eighteen days, they seemed to have consumed all that had

not become inaccessible in the soil, and began to show the same indications of defective supply as before. A new increment of combined nitrogen (excepting when in one or two instances it was added too late) caused a new increment of growth, greener colour, and a more vigorous appearance generally, to be soon followed by the recurrence of the pale colour, and so on. At each recurrence of these conditions, the plants must have passed, at a more advanced stage of growth than before, from the point of having an excess to that of having a deficiency of combined nitrogen.

A considerable range of conditions of growth was thus provided. Just after each addition, the plants must have been supplied with an excess of nitrogen in an available form, as was evinced in the formation of new shoots from the base or the nodes of the plants. But these new shoots, at first growing vigorously, soon suffered for want of a new supply; and in passing to this point, the newly-formed and vigorously-growing portion of the vegetable matter, would be in the condition assumed to be the most favourable to the assimilation of free nitrogen. This cycle of conditions, repeated several times during the growth of the same plant, and the experiment similarly conducted with a number of pots of plants of different kinds, with like results in all cases, afforded a wide range of circumstances assumed to be favourable to the assimilation of free nitrogen, which, however, has not taken place.

Table XX shows, that there remained in the soil, at the termination of the experiment, a greater or less amount of the combined nitrogen artificially supplied, yet the physiological evidence sufficiently showed the effect of each new addition; nor is it difficult to imagine that a few milligrammes of ammonia, intermingled with 1,500 or 1,600 grammes of soil (and pot), might become so distributed and absorbed, that a considerable proportion should remain inaccessible to the plant.

The Gramineous plants of 1858 were supplied with combined nitrogen at an earlier period of growth than those of 1857, and were not allowed to show such marked signs of deficiency before receiving fresh supplies.

Attention should be called, in passing, to the remarkable character of growth exhibited in some of the experiments.

Shortly after the addition of ammonia to the Gramineæ for the first time, the plants began to throw out new shoots from the base of the principal stem, as if they had first developed a stem commensurate with the limited amount of combined nitrogen con-

tained in the seed, and then, with the new supplies, been obliged to find vent for their activity in new directions. Some of the new shoots came forth close to the surface of the soil, some at the first, and some at the second nodes, as the drawings of the plants, given in plate 3, will show.

Another remarkable feature was the formation of roots at the second and third nodes above the ground, in the case of most of the Graminaceæ to which ammonia-salt was added. These roots came out around the node, and extended downwards, several reaching the soil from heights varying from $\frac{1}{2}$ to $1\frac{1}{2}$, or even 2 inches, and penetrating it to the bottom of the pot. The most marked instance was that of the barley, fig. 11, plate 3, given in more detail with special reference to these points, in fig. 16, plate 3. As the figures show, roots and new stems came from the same node, making the latter a new axis of growth, like the seed in the first instance. The original stems, below these nodes, did not increase much in size with the addition of ammonia, but the stems above the nodes became much larger than the portions below them, as did those of the new shoots.

Finally, in regard to the Graminaceæ: so long as an additional supply of combined nitrogen increased growth, so long must the physiological conditions have required available nitrogen, and therefore have been more or less favourable to the assimilation of free nitrogen if this were possible; but no such assimilation took place.

With regard to the other plants little more need be said than to direct attention to the columns of gain or loss of nitrogen. As already observed, the growth of the Leguminosæ was by no means so satisfactory as that of the Graminaceæ; and hence, the results relating to them apply to a more limited range of conditions of growth, and are, therefore, less conclusive against the possibility of the assimilation of free nitrogen in their case; though, so far as they go, they, as well as those with the buckwheat, tend to confirm those with the cereals. It should be remembered, too, that M. Boussingault experimented with a great many Leguminous plants, and generally succeeded in getting much more healthy growth than we were able to do, but in no case did he find any such gain of nitrogen as to lead to the conclusion that free nitrogen had been assimilated. We shall, ourselves, have additional evidence to bring forward on this subject on a future occasion.

In regard to the single experiment with clover, the results of which are given in Table XX, it may be mentioned that we failed

to get any growth in the experiment attempted without the addition of ammonia, and obtained comparatively little with it. Beans and peas also proved so sensitive under the conditions of experiment, and were so much less characteristically benefited by the supply of ammonia-salts, that it was obvious they suffered from other causes than a want of combined nitrogen. In subsequent experiments, however, nitrates have been found to be much more beneficial than ammonia-salts; as, indeed, they are when applied to Leguminous crops grown in the field.

It has already been pointed out that the beans grown in 1857 without extraneous supply of combined nitrogen, appropriated nearly four-fifths, and those grown in the same way in 1858 a larger proportion still, of the nitrogen of the seed sown. In contrast with these results, it may be mentioned that, in the experiment with beans, "1858 A" (see Table XX), there were 0.0523 gramme of nitrogen in the seed sown, and 0.0188 gramme supplied as ammonia-salt, making together 0.0711 gramme, of which the plants appropriated only 0.0401 gramme, or about one-fifth less than was supplied in the seeds alone; yet, it was in this experiment with Leguminous plants, that the addition of ammonia salt produced the most obvious effects on the growth.

From a review of the whole of the results of the direct experiments on the question of assimilation, it appears that the Gramineous plants grew the most healthily, and provided a wide range of conditions for the assimilation of free nitrogen; that the Leguminous plants were less healthy, and therefore provided a more limited range of conditions for assimilation; and that the growth of other plants was also less satisfactory than that of Gramineous ones. In all, the growth was more or less increased by the supply of combined nitrogen beyond that contained in the seed. The effect was the most marked with the Gramineous plants, their increase of dry vegetable substance due to the extraneous supply of combined nitrogen being 8, 12, and even nearly 30-fold, according to the amount provided. Yet, with 19 experiments with Gramineous plants, 6 with Leguminous ones, and some with plants of other descriptions, with such great variation in the amount and character of growth, and in the amount of combined nitrogen involved in the experiment in the several cases, in no instance have the results been such as to lead to the conclusion that there was an assimilation of free nitrogen.

The results of the whole enquiry may be briefly enumerated as follows:—

The yield of nitrogen in the vegetation over a given area of land, within a given time, especially in the case of Leguminous crops, is not satisfactorily explained by reference to the hitherto quantitatively determined periodical supplies of combined nitrogen.

Numerous experiments have been made by M. Boussingault, from which he concludes that free or uncombined nitrogen is not a direct source of the nitrogen of vegetation. M. G. Ville, on the other hand, concludes, from his results, that free nitrogen may be a source of a considerable proportion of the nitrogen of growing plants. The views, or explanations, of other experimenters, on this disputed point, are various and inconclusive.

It was found that the conditions of growth adopted in our own experiments on the question of the assimilation of free nitrogen, were consistent with the healthy development of various Graminaeous plants, but less so for that of the Leguminous plants experimented upon.

From the results of various investigations, as well as from other considerations, we think it may be concluded that, under the circumstances of our experiments, there would not be any supply to the plants of an unaccounted quantity of combined nitrogen, due either to the formation of oxygen-compounds of it under the influence of ozone, or nascent oxygen, or to that of ammonia under the influence of nascent hydrogen.

We have found that free nitrogen is given off in the decomposition of nitrogenous organic matter under certain circumstances. But, considering the circumstances of such evolution, and those to which the nitrogenous organic matter necessarily involved in experiments on the question of assimilation of free nitrogen is subjected, it may, we think, be concluded that there would be no loss of combined nitrogen from this cause in such experiments, excepting in certain cases when it might be pre-supposed.

Our experimental evidence, so far as it goes, does not favour the supposition that there would be any loss of combined nitrogen in the experiments on assimilation, due to the evolution of free nitrogen from the nitrogenous constituents of the plants during growth.

In numerous experiments with Graminaeous plants, grown both with and without a supply of combined nitrogen beyond that contained in the seed sown, in which there was great variation in the amount of combined nitrogen involved, and a wide range in the

conditions, character, and amount of growth, we have in no case found any evidence of an assimilation of free or uncombined nitrogen.

In our experiments with Leguminous plants, the growth was less satisfactory, and the range of conditions possibly favourable for the assimilation of free nitrogen was, therefore, more limited. But the results recorded with these plants, so far as they go, do not indicate any assimilation of free nitrogen. Since, however, in practice, Leguminous crops assimilate, from some source, so very much more nitrogen than Graminaceous ones, under ostensibly equal circumstances of supply of combined nitrogen, it is desirable that the evidence of further experiments with these plants, under conditions of more healthy growth, should be obtained.

Results obtained with some other plants are in the same sense as those obtained with Graminaceæ and Leguminosæ, in regard to the question of the assimilation of free nitrogen.

In view of the evidence of the non-assimilation of free nitrogen by plants under the wide range of circumstances provided in the experiments, it is desirable that the several actual or possible sources of *combined* nitrogen to plants should be more fully investigated, both qualitatively and quantitatively.

If it be established that the processes of vegetation do not bring free nitrogen into combination, it still remains not very obvious to what actions a large proportion of the existing combined nitrogen may be attributed.